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THE MICROBIAL ECOLOGY OF SULFUR TRANSFORMATIONS IN OYSTER POND, WOODS HOLE, MASSACHUSETTS

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THE MICROBIAL ECOLOGY OF SULFUR TRANSFORMATIONS IN OYSTER POND, WOODS HOLE, MASSACHUSETTS

by

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DEDICATION

This thesis is dedicated to my wife Trina, who through her love, patience, and understanding, offered encouragement to me throughout this endeavor.

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LIST OF ABBREVIATIONS

S‰ = salinity, ppt

Cl‰ = chlorinity, ppt

ppt = parts per thousand = g/liter or g/kg

ppm = parts per million = mg/liter or mg/kg

ppb = parts per billion - ug/liter or ug/kg

MPN = most probable number

cpm = counts per min

C = curies

mC = millicuries

μC = microcuries

ABSTRACT

THE MICROBIAL ECOLOGY OF SULFUR TRANSFORMATIONS
IN OYSTER POND, WOODS HOLE, MASSACHUSETTS

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FRANK W. BARVENIK

Oyster Pond is a small eutrophic coastal pond near Woods Hole, Massachusetts. The combined effects of a halocline and a summer thermocline lead to a stable stratified aquatic environment, containing sulfide and other reduced sulfur compounds in the hypolimnion.

The distribution of sulfate and sulfide in the water column suggests that the primary source of sulfide is dissimilatory sulfate-reduction. This conclusion is supported by enumeration results for dissimilatory and non-specific sulfate-reducing bacteria in the water column and in the sediment.

During the middle of the summer there is a net deposition of sulfides from the hypolimnion into the sediment. The resultant black muds contain an abundance of pyrite

in addition to acid-soluble and water-soluble sulfides.

As determined using S^{35}-sulfide, most of the oxidation of sulfide in the metalimnion is due to abiotic reactions, but photosynthetic and non-photosynthetic biological oxidation occurs as well. Soluble sulfur species, such as sulfite and thiosulfate, are important intermediates in the abiotic oxidation of sulfide.

Light and dark CO_2 fixation was determined using C^{14}-bicarbonate. Most primary productivity occurs in the epilimnion. A massive bloom of *Chlorobium*, as many as 5×10^7 cells/ml, occurs near the top of the sulfide zone in the summer. This photosynthetic flora accounts for CO_2 fixation of 10-30 mg C/m^3/hr and sulfide oxidation up to 4 mg S/liter/day.

A variety of non-photosynthetic sulfide-oxidizing microorganisms occur in the metalimnion. These organisms are responsible for oxidizing up to 4.5 mg sulfide-S/liter/day. A diverse flora of dark CO_2 fixing microorganisms is also present in this zone, fixing 18-27 mg C/m^3/hr during the summer.

Thiobacilli are present in the upper part of the sulfide zone. The maximum MPN of *Thiobacillus thioparus* was 4900/100 ml, whereas fewer of *T. thiooxidans* and *T. denitrificans* were observed. In terms of total numbers, dark CO_2 fixation, and sulfide oxidation, thiobacilli play a relatively minor role in this zone.

INTRODUCTION

Stratified aquatic ecosystems are suitable environments for the study of the natural sulfur cycle. The stratification of the water column, induced by vertical variations in salinity and temperature, has the effect of separating the hypolimnion from the freely circulating epilimnion. The hypolimnion can become a nutrient trap, and high concentrations of reduced sulfur compounds may accumulate near the bottom.

Aspects of the limnology and biogeochemistry of Oyster Pond have been studied by K. O. Emery and a number of other investigators from the Woods Hole Oceanographic Institution and the Marine Biological Laboratory. Its small size, convenience to laboratory facilities, and similarity to other coastal ponds, meromictic lakes, and anoxic marine basins such as the Black Sea and the fjords, make it an ideal environment for investigation.

It had been hypothesized by Egounov in 1898 that an intense flora of sulfur bacteria would occur in a thin layer near the top of the sulfide-containing zone of stratified aquatic environments. Conflicting reports have appeared in the literature regarding the occurrence, species composition, chemolithotrophic productivity, and sulfide oxidation of such a microbial enrichment.

The purposes of this investigation have been: 1. determine whether an enrichment of thiobacilli and other sulfur bacteria occurs at intermediate depths of Oyster Pond; 2. establish the physical, chemical, and biological conditions which lead to this enrichment; 3. evaluate the role of this flora of sulfur bacteria in terms of productivity; 4. determine the relative importance of biological and non-biological oxidation of sulfide; 5. determine the distribution and roles of various other microorganisms active in oxidizing and reducing sulfur compounds; 6. evaluate some aspects of the sulfur economy of the water column and sediments of Oyster Pond. Hopefully, this investigation will serve to clarify some of the quantitative aspects of the natural sulfur cycle in a stratified aquatic environment.

LITERATURE REVIEW

Principles and Examples of Aquatic Stratification

A stable stratified aquatic environment has an inherent resistance to mixing. This is due to a density difference (pycnocline) and work is required to mix the overlying lighter water with the underlying heavier water (Vallentyne, 1957; Harvey, 1963). In most freshwater lakes, the density stratification is due to a thermal difference between the surface waters and the deeper waters.

The prime reason for the thermal stratification is absorption of solar radiation at the surface (Ruttner, 1966). Wind-driven and other currents mix the heated water with cooler water from below (Vallentyne, 1957). This mixing, however, does not necessarily extend to the greatest depth. Depending on the local wind conditions, the degree of solar heating, the surrounding topography of the land, and the morphometry of the lake basin, a lake may achieve a characteristic thermal structure. The upper region of fairly uniformly warm, circulating water is known as the epilimnion, while cold, relatively undisturbed region below is known as the hypolimnion. The thermocline, or area of maximum temperature difference, lies between. This intermediate layer is also known as the metalimnion or the discontinuity layer (Hutchinson, 1957).

Most lakes in temperate latitudes, in which the stratification is primarily due to a thermocline, are not permanently stratified. Complete circulation occurs at least once a year; such a lake is called holomictic. Typical temperate lakes are uniformly cold in the early spring; at this time free circulation occurs. Then, as solar heating becomes more intense, a summer thermocline results. In the autumn, solar heating decreases, heat loss takes place primarily through radiation, and wind driven mixing intensifies, resulting in a fall circulation period. During the winter, ice may form on the surface, and the surface is colder than the rest of the lake (Hutchinson, 1957; Vallentyne, 1957; Ruttner, 1966).

Some lakes, however, are permanently stratified; such lakes are called meromictic. Even during the spring and fall when the temperature of the water column may be practically uniform, complete circulation does not occur. The deep stagnant waters are known as the monimolimnion while the overlying portion, in which circulation occurs periodically, is known as the mixolimnion. In most such lakes, the stability is due to increased content of dissolved salts in the monimolimnion. Thus, there is an intermediate layer with a density difference due to a salt gradient; such a layer is known as a halocline or a chemocline (Hutchinson, 1957; Vallentyne, 1957; Ruttner, 1966).

Meromixis can be due to a number of mechanisms. An ectogenic meromictic lake is one in which the halocline is caused by the catastrophic entrance of freshwater into a saline lake or salt water into a freshwater lake. Crenogenic meromixis is caused by the delivery of dense water from saline springs into the depths of a lake. Biogenic meromixis is due to the accumulation in the monimolimnion of sedimentary salts, thought to be released as a result of biological activity, either direct of indirect (Hutchinson, 1957).

An excellent example of a meromictic lake is Green Lake in Fayetteville, New York (Eggleton, 1956; Deevey, et al., 1963). This lake is only 3/4 miles long yet it has a depth of 59 m. It is protected from the wind by surrounding hills. The fact that the volume of outlet is much greater than the volume of the inlet implies the existence of subsurface springs, inlets, or seepages. This conclusion is also supported by the stratification of electrolytes; the conductivity is considerably higher in the monimolimnion than in the epilimnion. The waters contain a relatively high content of sulfate and carbonate. The result is that water below 20 m is permanently stagnant.

This lake illustrates one of the most important ramifications of meromictic lakes. The surface waters are well oxygenated while the bottom waters are permanently anoxic and contain up to 39.4 ppm sulfide (Deevey, et al., 1963). Holomictic lakes, especially the more productive ones, often develop anoxia in their hypolimnions as well, but this condition typically disappears during the periods of circulation; there are many examples of lakes of this type (Hutchinson, 1957; Ruttner, 1966). The mechanisms responsible for the development of anoxic conditions will be discussed below.

Marine waters are stratified as well. In tropical latitudes a permanent thermocline may be present at a depth below 100 m. Summer thermoclines are also present at depths of 10-50 m in the temperate latitudes. The surface water temperature in broad areas of the oceans often exceeds 27 C and higher temperatures are found in localized environments; the depths of the oceans, on the other hand, have temperatures below 4 C (Harvey, 1963).

According to Harvey (1963), the reduced eddy viscosity of the thermocline favors horizontal transport of the separated water masses. This often results in a change in salinity at the thermocline. In high latitudes, especially in areas of high run-off from the continents and from melting ice, the salinity of the upper part of the water column is lower than that in the deeper waters. The salinity in the upper layers in lower latitudes is usually greater than that in the deeper waters; this is primarily due to evaporation at the surface (Harvey, 1963).

In spite of their stratification, the deep waters of the oceans can hardly be called stagnant (Bowden, 1965). Strong circulation patterns exist in the world's oceans and there are areas where vertical transport (upwelling and convection) occur. In addition, turbulent mixing takes place. These processes replenish the depths with oxygenated water (Harvey, 1963).

There are broad areas of semi-tropical oceans, on the other hand, where an oxygen-minimum layer is found at intermediate depths. Oxygen concentrations lower than one ppm are common in this zone. The oxygen minimum layer is thoroughly discussed by Harvey (1963) and Richards (1965).

In certain semi-enclosed basins, where circulatory processes are restricted, anoxic conditions are found; such basins are typically separated from the sea by a relatively shallow sill. The density stratification may be due to a thermocline, a halocline or both.

There are a few tropical basins which are anoxic due entirely to the intense permanent thermocline. An example is the Cariaco Trench, a 1400 m deep depression in the continental shelf north of Venezuela (Richards and Vaccaro, 1956). The sill depth is 150 m and the water is anoxic below 375 m. Sulfide concentrations near the bottom are less than one ppm. The adjacent Gulf of Cariaco is intermittently anoxic (Richards, 1965). Another example of a permanently anoxic basin of this type is Kaoe Bay, Indonesia (Richards, 1965).

There are a number of offshore basins in which the circulation is limited due to the sill depth and the thermocline. These basins, such as those off the southern California coast and those in the Gulf of California, though not completely anoxic, have considerably reduced oxygen concentrations in their bottom waters (Emery and Rittenberg, 1952; Berner, 1964a).

Anoxic estuaries are most common in temperate regions where precipitation is heavy. In most estuaries, seawater is diluted by the entering freshwater, and there is a salinity gradient between the head and the mouth (Harvey, 1963). An intense vertical halocline is known to be present in a number of less typical estuaries.

The most extreme examples are the fjords, which are found along the coasts of northern Eurasia, northern North America, and southern South America (Ström, 1939; Richards, 1965). A fjord has been defined as "an elongated indenture of the coast line containing a relatively deep basin with a shallow sill at the mouth" (Saelen, 1967). It is generally thought that they were carved by an advancing glacier from an existing valley and the sill may consist of unconsolidated glacial till.

Lake Nitinat, a fjord on Vancouver Island, British Columbia, has been extensively studied (Richards, 1965; Richards, _et al._, 1965). Whereas it has an effective sill depth of only 4 m, the maximum depth is 210 m. A strong halocline exists above 20 m and the salinity of the bottom water is in the range of 31-32 ppt; a summer thermocline is also present. The water below 20-40 m is probably permanently anoxic and sulfide concentrations as high as 10 ppm were observed.

Other fjords, such as Saanich Inlet, British Columbia, are anoxic in the summer only, due to the instability of waters across the sill (Richards, 1965). Although not fjords, some of the basins of the Baltic Sea (especially the Gotland Basin) are similar. These basins are occasionally anoxic due to an intense halocline (Fonselius, 1963).

On the west coast of Japan there is an interesting series of lakes (Yoshimura, 1932). Lake Mikata consists entirely of freshwater. Its waters flow into Lake Suigetu and Hiruga, both of which contain seawater from Wakasa Bay in their deeper portions. As a result, Lake Suigetu is permanently anoxic and Lake Hiruga is similar except that it mixes completely during severe winters. Lake Kugusi is shallow and probably is a simple estuary.

The Black Sea, an extremely large, deep tributary of the Mediterranean Sea, is the best known anoxic basin (Caspers, 1957; Zenkevich, 1963). It has a mean depth of 1271 m and a maximum depth of 2245 m. The Bosporus and the Dardanelles form a narrow, shallow sill. Like most temperate estuaries the freshwater discharge is greater than the influx of saline water; a pronounced halocline exists at 100-200 m. A thermocline adds to the stability of the water column during the summer. The sulfide content of the bottom waters ranges up to about 10 ppm. The sea of Azov, attached to the northern part of the Black Sea, is an estuary of the Don River. This extremely productive water body is intermittently anoxic.

Some hypersaline waters, like the Sivach or Putrid Sea, the Dead Sea, and a number of salt lakes are intermittently or permanently anoxic (Zenkevich, 1963; Neev and Emery, 1967; Butlin and Postgate, 1954).

Conditions Leading to Anoxia and Sulfide Production in Stratified Aquatic Environments

On this planet, at this time, virtually all the organic matter in natural environments is derived ultimately from biological photosynthesis. In aquatic environments, organic matter can be produced "in situ" in the photic zone (autochthonous) or it may be derived from exogenous sources (allochthonous).

There are numerous examples of allochthonous organic matter contributing to the natural economy of aquatic environments. In their e tensive survey of the Highland Lake District of northeastern Wisconsin, Birge and Juday (1934) found that many lakes received substantial contributions from external sources such as leaves, humus, marshes, and peat. This material was included in both the soluble and the suspended organic matter and accounted for 38.8 - 75.2% of the total organic matter of the lakes. A major source of allochthonous organic material in many aquatic environments is domestic and industrial pollution (Mackenthun and Ingram, 1967).

In the sea, the most important primary producers of autochthonous organic matter are the phytoplankton. The attached algae are quantitatively important only in the littoral zone which comprises a small proportion of the total oceanic area (Raymont, 1963). In fresh waters, on the other hand, littoral production may be quantitatively important. The longer the shoreline and the shallower the slopes in relation to the total area of the lake, the greater the importance of littoral production (Ruttner, 1966). In fresh waters rooted plants with their associated periphyton are the most significant producers in the littoral zone, while the phytoplankton are important in both the littoral and pelagic zones.

Green plant photosynthesis is an oxygen-producing reaction; it represents a major mechanism for replenishment of waters with oxygen. As a result of photosynthetic activity, productive waters are often supersaturated with oxygen. Photosynthetic activity also depletes the water of CO_2, resulting in a rise in pH. Concurrent with photosynthesis, dissimilation of organic matter is also taking place in the water column. The process is mediated by plants, animals, and bacteria. It is important to note that dissimilatory processes result in the production of CO_2 and the consumption of oxygen.

The fact that photosynthetic assimilation of organic matter is limited by light and dissimilation of organic matter is not limited by light is important in the chemical stratification of aquatic environments. In such environments there is a depth, the compensation point, at which assimilation and dissimilation are equal (Ruttner, 1966). The actual depth of the compensation point depends on the local flora and on the transparency of the water. The compensation point can be estimated to be the depth to which about 1% of incident visible radiation penetrates (Ruttner, 1966). Above the compensation point there is a net production of organic matter, consumption of CO_2, and production of oxygen. Below this point, there is a net consumption of organic matter and oxygen and production of CO_2.

Thus, the dissimilation of organic matter is the primary mechanism in the development of anoxia. The greater the amount of organic matter to be decomposed, the more likely that the oxygen will be depleted (Richards, 1965). It is, therefore, more likely that eutrophic waters become anoxic than oligotrophic waters.

Stoichiometric models for the dissimilation of organic matter in aquatic environments have been made (Redfield, 1958; Richards, 1965; Richards, *et al.*, 1965). These models have been based on the composition of average plankton material, in which the atomic ratio is C:N:P: = 106:16:1 (Fleming, 1940). Richards (1965) then considered that the average organic matter in the sea might have the empirical formula $(CH_2O)_{106}(NH_3)_{16}H_3PO_4$. In oxygenated water, the complete oxidation of this organic matter and the ammonia released can be represented:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\ O_2 \longrightarrow$$
$$106\ CO_2 + 122\ H_2O + 16\ HNO_3 + H_3PO_4 \quad \text{(Richards, 1965).}$$

Ratios of $(O_2{:}CO_2{:}NO_3^-{:}PO_4^{=})$ can be derived from this equation, representing the changes which would occur during complete decomposition of organic matter in the marine environment. According to Richards, *et al.* (1965), despite the many possible errors in the assumptions, the model has been tested a number of times and appears to be a reasonable approximation in oxygenated waters.

While the above model can apply only where dissolved oxygen is present, a model has been proposed by Richards (1965) for the dissimilation of organic matter in anoxic marine environments. When most of the dissolved oxygen has been consumed, denitrification is likely to take place. In this process, nitrate serves as terminal electron acceptor, instead of oxygen, and molecular nitrogen is the ultimate product. Richards represents denitrification by the following equation (assuming oxidation of the ammonia released):

$$(CH_2O)_{106}(NH_3)_{16}\ H_3PO_4 + 94.4\ HNO_3 \longrightarrow$$
$$106\ CO_2 + 55.2\ N_2 + 177.2\ H_2O + H_3PO_4$$

The nitrogen isotope data of Richards and Benson (1961) suggests the occurrence of denitrification in the Cariaco Trench and in Dramsfjord, Norway.

When the oxygen and nitrate are consumed, sulfate reduction can take place. In this process, sulfate serves as terminal electron acceptor during the oxidation of organic matter. The dissimilation of organic matter by sulfate reduction has been represented by Richards by the equation:

$$(CH_2O)_{106}\ (NH_3)_{16}\ H_3PO_4 + 53\ SO_4^{=} \longrightarrow$$
$$106\ CO_2 + 53\ S^{=} + 16\ NH_3 + 106\ H_2O + H_3PO_4$$

Richards, *et al.* (1965) used ratios derived from these equations to test the model in an anoxic fjord, Lake Nitinat. They found a good correlation between the ammonia and sulfide concentrations and the atomic ratio between them was not significantly different from the predicted ratio of 3.3:1. While this leads to a certain amount of confidence in the model, the other predictions were more poorly supported. The correlations were either not linear or the slopes differed significantly from the predicted ratios.

Methanogenesis is often regarded as an alternative mechanism for organic matter dissimilation (Richards, _et al._, 1965; Postgate, 1968). In this process, CO_2 is the terminal electron acceptor for the oxidation of organic matter. Methane gas has been demonstrated in anoxic waters and sediments (Kanwisher, 1962; Richards, _et al._, 1965). Deevey, _et al._, (1963) have deduced from carbon fractionation data that methane is produced biologically in anaerobic sediments.

An important ramification of anoxic conditions in aquatic environments is the accumulation of nutrients in the anoxic zone (Richards, 1965). The concentrations of ammonium, phosphate, carbonate and silicate are very much greater in the anoxic zone than in the overlying oxygenated water. Redfield (1968) considered such basins to be "nutrient traps". This accumulation appears to be the direct result of mineralization of organic matter produced in the photic zone. When such nutrients are carried back to the surface, as in turnovers and upwelling, a marked increase in productivity can be expected (Harvey, 1963; Ruttner, 1966).

While it might be expected that significant amounts of organic matter might be removed from circulation in anoxic zones by sedimentation, the decomposition of organic matter in the water column of the Black Sea is apparently almost complete (Kriss, 1963). The amount of organic matter in sediments of the Black Sea is similar to that in oxygenated marine basins (Zenkevich, 1963). This implies that denitrification, sulfate reduction, and methanogenesis are effective methods for dissimilation of organic matter.

Production of Sulfide

The dissimilatory sulfate-reducing bacteria represent a unique group of organisms. Their taxonomy has been discussed recently by Campbell and Postgate (1965) and Postgate and Campbell (1966). They are members of two morphologically distinct genera, *Desulfovibrio* and *Desulfomaculum*. The former genus consists of non-sporulating, gram-negative vibrios with polar flagella, whereas the latter genus includes gram-negative rods with terminal or subterminal endospores and with peritrichous flagella. Some members of *Desulfomaculum* are thermophilic.

These bacteria are strictly anaerobic and Baas Becking *et al*. (1960) have stated that the absolute limits of their environmental ranges are pH 4.15-9.92 and Eh +115-(-450) mV. Various strains of the dissimilatory sulfate-reducing bacteria will grow in either fresh or salt water. Their carbon metabolism has been reviewed by Postgate (1959).

They have been reported to be capable of growth on a variety of organic materials, but Postgate points out that the spectrum of carbon compounds metabolized is influenced by the Eh of the medium and the presence of yeast extract. He also notes that cultures used in some reports may not have been pure. The metabolism of 3- and 4- carbon compounds has been studied in some detail.

Growth of dissimilatory sulfate-reducing bacteria at the expense of hydrogen oxidation has also been reported by a number of investigators (Postgate, 1959), but complete chemolithotrophy apparently does not occur (Mechalas and Rittenberg, 1960).

Desulfovibrio and *Desulfomaculum* spp. are capable of using a variety of sulfur compounds as terminal electron acceptors. While sulfate-reduction is regarded as the most important reaction, the organisms can also reduce thiosulfate, tetrathionate, sulfite, and colloidal sulfur (Postgate, 1959). Partial reduction of sulfate to sulfur has also been reported. The pathway of sulfate-reduction was reviewed by Peck (1962) and Trudinger (1969).

Under certain conditions dissimilatory sulfate-reducing bacteria are capable of fractionating the stable isotopes of sulfur; their activity has the effect of enriching the sulfate in the heavier isotope (S^{34}) and enriching the sulfide in the lighter isotope (S^{32}) (Jones and Starkey, 1957). This ability to fractionate the sulfur isotopes has been used by a number of investigators as an index of activity of sulfate-reducing bacteria (Macnamara and Thode, 1951; Jones, et al., 1956). In this way, Deevey, et al., (1963) found fractionation factors of 1.020 in the hypolimnion of Linsley Pond, Connecticut, and 1.0575 in the monimolimnion of Green Lake, New York. The unusually great fractionation in the latter lake was considered to be evidence that the stagnant waters of this meromictic lake constitute a nutrient trap.

A variety of procaryotic and eucaryotic organisms are capable of sulfate-reduction for assimilatory purposes. Peck (1962) and Trudinger (1969) review the pathways of reduction of sulfate and incorporation into cysteine. When assimilatory sulfate-reduction is followed by protein decomposition and release of sulfide the process has been called non-specific sulfate-reduction (ZoBell, 1946). The property is widespread in such genera as Aerobacter, Bacillus and Proteus (LaRiviere, 1966). While the sulfur containing amino acids cysteine, cystine, and methionine, constitute the bulk of the organic sulfur in cells, sulfur also appears in a number of other types of organic compounds. The occurrence, transformations, and dissimilation of organic sulfur compounds was reviewed by Starkey (1964).

Dissimilatory sulfate-reducing bacteria are known to be widely distributed in anaerobic marine and freshwater and sediments (ZoBell and Rittenberg, 1948; Ruttner, 1966). Some workers have attempted to determine the relative importance of assimilatory and dissimilatory sulfate-reducing bacteria in such environments. Kriss (1963) pointed out that there is no evidence to show the prevalence of one group or the other in the Black Sea. Ivanov and Terebkova (1959a) reported 0-10 dissimilatory sulfate-reducing bacteria/ml and 200-1000 non-specific sulfate-reducing bacteria/ml in Lake Solenoe, a shallow stratified lake. The numbers in the sediments were higher: 1000-100,000 dissimilatory sulfate-reducing bacteria/g and 1600-6000 non-specific sulfate-reducing bacteria/g.

Gunkel and Oppenheimer (1963) incubated some shallow sediments from the Gulf of Mexico and the North Sea in opaque tubes "in situ" and under laboratory conditions for several weeks. In most cases the increase in sulfide-S was considerably greater than the decrease in sulfate-S. This would imply that there is another source of sulfide, presumably non-specific sulfate reduction. It is interesting to note that the numbers of non-specific sulfate-reducing bacteria were considerably higher (1-2 logs) than numbers of the other group. Skopintsev, et al. (1964) reports data from experiments conducted with samples from the Black Sea. Concentrated plankton samples were added to water samples which were incubated in the dark under aerobic and anaerobic conditions. In these experiments, the increase in sulfide-S agreed with the decrease in sulfate-S.

The use of radioactive sulfur (S^{35}) has provided a useful technique for the estimation of "in situ" sulfate-reduction rates. In this technique, labelled sulfate is incubated with the water or sediment sample and the appearance of the label in the sulfide fraction is determined (Ivanov, 1959). Ivanov and Terebkova (1959a and b) observed sulfate-reduction rates of 0.9-19 mg H_2S/liter/day in bottom deposits of Lake Solenoe in the summer and rates of 0.0017-1.404 mg/liter/day in the winter. Sorokin (1962) observed sulfate-reduction rates of 0.027-2.31 mg H_2S/liter/day in surface sediment samples from the Black Sea. He later (1964) observed that sulfate-reduction occurs actively in the water column of the Black Sea in two zones, a layer about 150 m thick near the upper boundary of the anoxic zone and a layer near the bottom; the maximum rates observed in the water column were about 0.007 mg H_2S/liter/day. The use of a similar technique for estimating the rates of non-specific sulfate-reduction has not been reported.

Sulfide can also originate from exogenous sources such as vulcanism, springs, and pollution. For example, Lake Sernoye, U.S.S.R. is fed by sulfide-containing mineral springs (Kuznetsov, _et al._, 1963). Sulfite waste-liquor from pulp mills can be a source of sulfide as well as sulfite (Black, 1960; Eldridge, 1960).

Solubility and Volatilization of H_2S

The ionization of H_2S was considered in detail by Hutchinson (1957). H_2S is freely soluble in water; the solubility at 1 atm at 25 C is 0.102M (3.47 ppt). It behaves as a weak acid, ionizing as follows (Borchert, 1965).

$$H_2S \rightarrow H^+ + HS^- \qquad K_1 = 0.91 \times 10^{-7} \text{ at } 18\text{ C}$$

$$HS^- \rightarrow H^+ + S^= \qquad K_2 \cong 1.0 \times 10^{-15}$$

The relative molar concentrations of the various species in water with a metal ion concentration of 10^{-5} M are given by Baas Becking (1925):

pH	H_2S	HS^-	$S^=$
5	2.2×10^{-3}	2.0×10^{-5}	2.0×10^{-15}
6	1.2×10^{-4}	1.1×10^{-5}	1.1×10^{-14}
7	1.1×10^{-5}	1.0×10^{-5}	1.0×10^{-13}
8	1.0×10^{-6}	9.1×10^{-6}	9.1×10^{-13}
9	2.2×10^{-8}	2.0×10^{-6}	2.0×10^{-12}

Thus, at pH 7 approximately equal amounts would be present as H_2S and HS^-, while at pH 8 most would be present as HS^-.

Eriksson (1961) noted that H_2S is an extremely volatile gas and, except for the overlying layer of oxygenated water, some escape into the atmosphere would be likely.

Sedimentation of Sulfides

The metallic sulfides are quite insoluble and according to Hutchinson (1957) the sulfide concentration in solution is controlled by the precipitation of ferrous sulfide. He discusses in detail the solubility of other metallic sulfides as well.

It is likely that the low oxidation-reduction potential of anoxic waters results in lowered oxidation states of cations (Richards, 1965). Thus, while iron is mostly in the ferric state (+3) in oxygenated water, existing as insoluble ferric hydrates and complexes, it is often present as soluble ferrous ion (+2) in anoxic water. While the maximum soluble iron content of the Pacific is about 5.5 ppb (Lewis and Goldberg, 1954), Richards (1965) observed concentrations as high as 18 ppb in the Cariaco Trench. Skopintsev, et al (1964) observed concentrations up to 20 ppb in the Black Sea. Borchert (1965) pointed out that the leaching of iron from sediments is favored by a low pH, thus the relatively low pH characteristic of the intermediate waters ("CO_2 zone") of anoxic basins and lakes would increase the availability of iron.

The form in which the various iron ore minerals are precipitated from aquatic environments is influenced by the Eh and sulfide concentration (Borchert, 1965). While goethite (FeOOH) may be formed in oxygenated sediments, siderite ($FeCO_3$) may be formed under intermediate conditions, and pyrite (FeS_2) may be formed in sediments with a low Eh and a high sulfide content.

The iron sulfides may precipitate from solution in the water column or within the sediments. There are a number of iron sulfide minerals, differing in their physical and chemical properties (Vallentyne, 1963; Berner, 1964).

A possible sequence was presented by Emery and Rittenberg (1952). Sulfide would be originally precipitated as an amorphous form, hydrotroilite ($FeS \cdot nH_2O$), which would be subsequently crystallized to the transitional form, melnikovite (FeS_2). These two forms are black colored and acid soluble. In acidic environments rhombic FeS_2 (marcasite) would form slowly, while cubic FeS_2 (pyrite) would form in neutral or alkaline environments. Marcasite and pyrite are brassy yellow, opaque, and metallic, and are quite stable. This theory is supported by the appearance of pyrite or marcasite in offshore, slowly deposited sediments (green muds), while in areas of rapid deposition, such as bays, harbors, and estuaries, the black acid-soluble sulfides are found (Emery and Rittenberg, 1952). This data suggests that the latter sediments are being deposited too rapidly for alteration of the melnikovite to pyrite and marcasite to occur.

Emery and Rittenberg (1952) suggested another alternative. They noted that while many black muds are high in acid-soluble forms and low in free H_2S, the green muds are often high in free H_2S and low in the acid-soluble forms. This suggests that the form that the sulfides appear in is dependent on the availability of iron. Emery and Rittenberg suggested that hydrotroilite may not be an intermediate in slow deposition areas.

The sulfur in FeS_2 is in a higher oxidation state than in FeS. Baas Becking (1956) presented evidence for the existence of disulfhydryl iron ($Fe(SH)_2$ or $FeSH{\cdot}H_2S$), which could be oxidized to form pyrite:

$$Fe(SH)_2 + A \longrightarrow FeS_2 + H_2A$$

where A may be sulfur, ferric hydroxide, organic matter, or oxygen. An alternative mechanism was presented by Berner (1964a):

$$FeS + H^+ \longrightarrow HS^- + Fe^{++}$$

$$Fe^{++} + S^o + HS^- \longrightarrow FeS_2 + H^+.$$

Berner proposed that this reaction sequence could occur at the sediment water interface of the basins in the Gulf of California, where an abundance of all the above reactants were found. This could explain the lack of acid-soluble forms in such sediments. The observation of microfossils in pyrite suggests that microorganisms may play a role in pyrite formation (Love, 1957).

Vallentyne (1963) observed abundant pyrite spherules in almost all fine-grained freshwater sediments containing organic matter and reduced sulfur compounds. Of two meromictic lakes examined, Green Lake, New York, and Längsee, Austria, they were found only in the sediments of the latter. These spherules were often found within intact remains of higher plants and animals (plant cells, pollen grains, etc.). In Little Round Lake, Ontario, about 450,000 spherules (2-100 μ in diameter) were found/g dry weight of sediment; this represents about 1600 ppm by weight as pyrite-S.

Nriagu (1968) found that most of the sulfur in sediments of the eutrophic Lake Mendota, Wisconsin, was in the form of acid-soluble sulfides. Sugawara, et al. (1953) reported high concentrations of "unidentified S" in freshwater, brackish, and marine sediments. This "unidentified S" was later shown to consist largely of pyrite (Sugawara, et al., 1954).

Dissimilatory sulfate-reduction often appears to be the primary source of sulfide in the iron sulfide minerals (Emery and Rittenberg, 1952; Berner, 1964a). This is probably true in environments where the sulfate content is high and the organic content is relatively low. On the other hand, Nriagu (1968) suggested that as much as 45% of the sulfide in Lake Mendota sediments may have originated through non-specific sulfate-reduction.

Oxidation of Reduced Sulfur Compounds

Sulfide and other reduced sulfur compounds may be oxidized abiotically or biologically. The abiotic oxidation of sulfide is complex; there are several intermediates and the reaction sequence may be influenced by a number of variables. Bulfer, et al. (1936) noted that the presence of organic substances, such as glycerol and carbitol, inhibit the oxidation of Na_2S solutions. Krebs (1929) observed the catalytic effect of the first transition series metals (Mn, Ni, Co, Fe, Cu) on abiotic oxidation of various sulfides. The catalytic effect of iron was also observed by Wheatland (1954). Iya and Sreenivasaya (1944) suggested that reactive nitrite may serve as an oxidizing agent in the liberation of sulfur from sulfide.

The results of Skopintsev, et al. (1964) using low concentrations of O_2 and H_2S suggest that abiotic oxidation occurs in two stages:

$$H_2S \xrightarrow{O_2} S^o \xrightarrow{O_2} H_2SO_4,$$

following first-order kinetics. Avrahami and Golding (1968) also studied sulfide oxidation at low concentrations in aqueous solutions. Their results suggest that the reaction sequence is far more complex than above:

$$HS^- + 3/2\ O_2 \longrightarrow SO_3^= + H^+$$

$$SO_3^= + 1/2\ O_2 \longrightarrow SO_4^=$$

$$SO_3^= + HS^- + 1/2\ O_2 \longrightarrow S_2O_3^= + OH^-$$

$$S_2O_3^= + 1/2\ O_2 \longrightarrow SO_4^= + S^\circ$$

The first reaction is the initial rate-limiting step, and sulfite is then rapidly removed by further oxidation to sulfate or by production of thiosulfate. Oxidation of thiosulfate and sulfur proceeds slowly. They found that the oxidation rate is enhanced by increased pH, temperature, and O_2:N_2 ratio in the gas mixture. The results of Abel (1956) and Cline and Richards (1969) also suggest that soluble intermediates, such as H_2SO_2, $H_2S_2O_4$, $SO_3^=$, and $S_2O_3^=$, are important in the reaction sequence. While Avrahami and Golding (1968) observed that oxidation was close to first-order with respect to sulfide when other variables remained constant, Cline and Richards (1969) observed mixed second-order kinetics after the first few hr of reaction. Kester and Richards observed that the ultimate produce depends on the oxygen concentration in the water (Richards, 1965).

Chemolithotrophic sulfur bacteria (colorless sulfur bacteria) are capable of utilization of reducing power, obtained from the oxidation of reduced sulfur compounds, in order to reduce CO_2 to form cellular organic matter. While a number of groups of bacteria, both multicellular and unicellular, have been called chemolithotrophic sulfur bacteria, many of these organisms have questionable status.

Beggiatoa has long been thought to be a chemolithotrophic sulfur bacterium. This filamentous organism is capable of depositing elemental sulfur intracellularly when growing in the presence of sulfide; when the supply of sulfide is removed, the sulfur globules gradually disappear, presumably by oxidation to sulfate (Winogradsky, 1887).

Beggiatoa isolates were shown subsequently to be capable of heterotrophic growth (Faust and Wolfe, 1961; Scotten and Stokes, 1962). Burton and Morita (1964) demonstrated the beneficial effects of catalase when the organism is grown in the absence of sulfide. This would suggest that *Beggiatoa* produces peroxide which is eventually lethal, and that catalase or sulfide are capable of reducing the peroxide and allowing maximal growth. Burton and Morita admitted the possibility that the enrichment technique commonly employed (hay enrichment) may select for non-chemolithotrophic strains. Pringsheim and Kowallik (1964) observed that certain strains can develop on strictly inorganic media in the presence of low quantities of sulfide, and that without CO_2, no growth is observed. Some strains are apparently facultative chemolithotrophic sulfur bacteria.

Whether or not it is truly a chemolithotroph, *Beggiatoa* is capable of oxidizing sulfide, at least as far sulfur. *Beggiatoa* is widely distributed, especially in stagnant fresh and salt water containing hydrogen sulfide (Breed, *et al.*, 1957).

Included in the family Beggiatoaceae are three other genera of supposed sulfur bacteria, Thiospirillopsis, Thioploca, and Thiothrix (Breed, et al., 1957). Like Beggiatoa these organisms consist of gliding trichomes and contain sulfur globules when growing in the presence of sulfide; they are distinguished from Beggiatoa on the basis of their morphology.

Thioploca has been studied in some detail by Kolkwitz (1955) and by Maier and Murray (1965). The latter authors compared a strain obtained from the mud of western Lake Erie with cultures of Beggiatoa; they found their morphology to be similar but distinct. Thioploca differs primarily by consisting of several trichomes enclosed in a common sheath.

Rodina (1963) microscopically examined the detritus of several lakes for sulfur bacteria; as many as 1.8×10^9/g of wet detritus were observed. He observed various Beggiatoa and Thiothrix species, as well as photosynthetic sulfur bacteria. Thiothrix is similar to Beggiatoa, except that it is attached to a substrate by a holdfast. Thiospirillopsis, an organism with coiled trichomes, was observed by Lackey (1960) in a freshwater spring.

Lackey and Lackey (1961) described a new sulfur bacterium, Thiodendron mucosum, which has an unusual morphology. It consists of rod-shaped non-motile cells forming dendroid colonies in a jelly-like matrix. It was found in two aquatic environments in Florida.

Whether these multicellular forms are distinct genera is still questionable and will remain questionable until they are grown in axenic culture. Cultural studies may also be able to answer the question of whether they are actually chemolithotrophic. Presently, the only evidence for their inclusion among the sulfur bacteria is the intracellular deposition of sulfur. It should be noted such organisms as *Sphaerotilus natans*, *Leucothrix*, and even several yeasts and fungi, will deposit sulfur when exposed to hydrogen sulfide (Skerman, Dementjev, and Skyring, 1957; LaRiviere, 1965). This may represent a detoxification mechanism in such organisms.

Lackey (1960) observed many aquatic samples for the presence of colorless sulfur bacteria and attempted to determine the environmental factors governing their natural distribution. Among the factors which affect their distribution are light, temperature, barometric pressure, osmotic pressure, pH, and physical and chemical substrates. He was able to distinguish between 22 species of multicellular and unicellular forms, entirely on the basis of their morphology.

Most of the genera of unicellular sulfur bacteria are as questionable as the filamentous sulfur bacteria. Included in this group are *Achromatium*, *Thiobacterium*, *Macromonas*, *Thiospira*, *Thiovulum*, and *Thiobacillus* (LaRiviere, 1965). Like many of the filamentous forms, many of these organisms probably should be placed among the colorless blue-green algae (Pringsheim, 1949). They are typically included among the sulfur bacteria because they are found in environments containing sulfide, and because under these conditions they deposit sulfur intracellularly. Of the unicellular colorless sulfur bacteria, only *Thiovulum* and *Thiobacillus* have been studied in axenic culture.

Thiovulum is a member of the Thiobacteriaceae (Breed, *et al.*, 1957). The cells are spherical to ovoid, 5-25 μ long, and rapidly motile by peritrichous flagella; they divide by longitudinal fission (DeBoer, *et al.*, 1961). *Thiovulum* is extremely chemotactic and is specifically attracted to areas containing low concentrations of both dissolved oxygen and sulfide (LaRiviere, 1965). The cells contain inclusions of elemental sulfur. Since *Thiovulum* can only be grown under these conditions and does not appear to require organic compounds, it is currently regarded as a chemolithotrophic sulfur bacterium (LaRiviere, 1965). According to Skerman (1965), they are typically found in seawater in the vicinity of decomposing organic matter liberating H_2S, where they form a thin film of growth in a zone of critical sulfide and oxygen concentrations.

Thiobacillus is also a member of the Thiobacteriaceae. Unlike the other colorless sulfur bacteria, this genus has been studied in considerable detail for many years, and its chemolithotrophy is well established. *Thiobacillus* Beijerinck is characterized by small, gram-negative, rod-shaped bacteria, possessing single polar flagella when motile. The genus includes obligate and facultative chemolithotrophic members, deriving their energy from the oxidation of several reduced sulfur compounds. They do not deposit elemental sulfur intracellularly (Breed, *et al.*, 1957).

There are a number of distinct species of *Thiobacillus*, but the breakdown in Bergey's Manual of Determinative Bacteriology, 7th Ed. (Breed, *et al.*, 1957) is probably invalid. The taxonomy of the species of *Thiobacillus* has been considered more recently by Unz and Lundgren (1961); Hutchinson, Johnstone, and White (1965, 1966 and 1967); Sokolova and Karavaiko (1968); and Rittenberg (1969).

Thiobacillus thioparus is the type species of the genus. This organism is neutrophilic, but it is capable of dropping the pH to 3.5-4.5 when growing in thiosulfate media. It is an obligate chemolithotroph and under certain conditions it can oxidize sulfide, sulfur, thiosulfate, tetrathionate, and, to a lesser extent, trithionate, dithionate and hyposulfite (Sokolova and Karavaiko, 1968).

There are several other thiobacilli which are very similar to *T. thioparus*. *T. neapolitanus* (*Thiobacillus* X) appears to be somewhat more acid-tolerant than *T. thioparus* (Hutchinson, Johnstone and White, 1965); its ability to form tetrathionate during thiosulfate oxidation is not considered as a distinctive characteristic by Sokolova and Karavaiko (1968) since *T. thioparus* will also form tetrathionate under certain conditions (Vishniac and Santer, 1957). *T. thiocyanoxidans* differs from *T. thioparus* in its additional capability to utilize thiocyanate as sole source of energy, carbon, and nitrogen (Vishniac and Santer, 1957). A halophilic strain of *T. thioparus* was reported by Keller (1969).

T. thiooxidans is also an obligate chemolithotroph, capable of oxidizing thiosulfate, sulfur and possibly sulfide (Sokolova and Karavaiko, 1968). It is an extreme acidophile, its optimum pH being 2.0-3.5; during growth especially on sulfur, it drops the pH of its medium to 0.5 or below (Breed, et al., 1957). T. concretivorus is probably a strain of T. thiooxidans (Vishniac and Santer, 1957; Sokolova and Karavaiko, 1968). T. ferrooxidans, like T. thiooxidans, is an acidophile. However, it is capable of growth on ferrous iron and in addition to some sulfur compounds; it appears to be quite similar to Ferrobacillus ferrooxidans, and F. sulfooxidans, the three strains differing only in range of substrates (Unz and Lundgren, 1961; Sokolova and Karavaiko, 1968).

T. denitrificans is capable of growth anaerobically, producing gaseous nitrogen from nitrate (Baalsrud and Baalsrud, 1954). Like many denitrifying bacteria it is also capable of aerobic growth. While growing aerobically, it is quite indistinguishable from T. thioparus. According to Hutchinson, Johnstone, and White (1967), T. intermedius and T. thermophilica are also capable of anaerobic growth.

Rittenberg (1969) compared the various examples of facultative autotrophic, chemolithotrophic, heterotrophic, and mixotrophic bacteria, and attempted to classify them in a logical manner. He concluded that the modes of carbon assimilation and energy generation are independent and occur in all possible combinations. He defined obligate chemolithotrophs as those which derive their energy from oxidation of inorganic materials, have no mechanisms for obtaining energy from organic compounds, and which utilize the ribulose diphosphate cycle as their basic mechanism for carbon assimilation. Chemolithotrophic heterotrophs, such as T. perometabolis and Desulfovibrio desulfuricans, were defined as those which lack the ribulose diphosphate cycle (consequently, CO_2 fixation can play only a secondary role in their carbon assimilation), but which may be capable of utilizing energy obtained from inorganic oxidations. All possible combinations between were grouped as mixotrophs, or facultative autotrophs. T. novellus and T. intermedius were included in this group. These two strains differ in the degree that their thiosulfate-oxidizing systems are repressed by organic matter, their final pH in thiosulfate media, and their relative ability to grow on certain single organic compounds; both are capable of growth in thiosulfate media and in certain complex organic media (Adair and Gundersen, 1969b; Rittenberg, 1969).

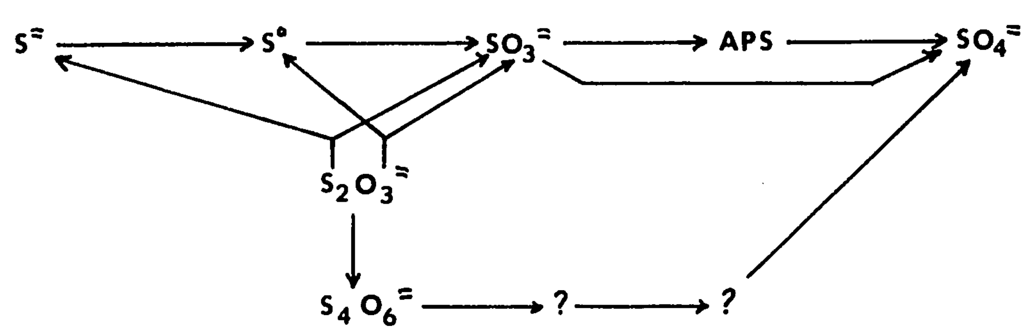
This classification is further complicated by the reports that such "obligate chemolithotrophs" as Thiobacillus thiooxidans are capable of limited chemoorganotrophic growth (Borichewski and Umbreit, 1966; Rittenberg, 1969). Exogenous organic compounds may also be taken up by thiobacilli during chemolithotrophic growth (Rittenburg, 1969).

Egorova and Deryugina (1963) isolated a thermophilic, spore-forming organism, capable of growth by oxidizing sulfide, sulfite, thiosulfate, and hyposulfite. They called the organism T. thermophilica, although its morphology differs from the other members of the genus.

Many other species of thiobacilli have been described, such as *T. coproliticus*, *T. beijerinckii*, *T. nathansonii*, *T. thermitanus*, *T. lobatus*, *T. umbonatus*, *T. crenatus*, and *T. trautweinii*; most of these are currently regarded as strains of established species (Vishniac and Santer, 1957). *T. trautweinii*, however, should not be included in the genus at all. This organism is incapable of growth on mineral media, but does slowly oxidize thiosulfate to tetrathionate with a rise in pH while growing on organic media; it probably should be included in the genus *Pseudomonas* (Starkey, 1935; Trudinger, 1969).

There is extensive literature on the physiology of the thiobacilli. This literature was reviewed by Vishniac and Santer (1957), Peck (1965), Vishniac and Trudinger (1962), Sokolova and Karavaiko (1968), Rittenberg (1969), and Trudinger (1969). There is considerable controversy concerning the intermediary metabolism, response to organic matter, phosphorylation and electron transport, extracellular products, and pathways of oxidation of sulfur compounds of this genus.

The oxidation of sulfur compounds by the thiobacilli is summarized below (Trudinger, 1969):



where APS is adenosine phospho sulfate.

This sequence illustrates only the major reactions and not every member of the genus is able to perform each step.

Sulfide may be oxidized by a number of thiobacilli. However, now all metallic sulfides are oxidized; the heavy metal sulfides are quite insoluble and oxidation may involve intimate contact between the cells and the mineral (Sokolova and Karavaiko, 1968). Sulfur, thiosulfate, and polythionates have been reported as intermediates and there is little agreement on the pathway of sulfide oxidation. There is reason to doubt whether the energy from sulfide oxidation is utilized; sulfide may not be utilized until after its chemical conversion to other metabolizable compounds (Trudinger, 1969).

Intimate contact between _T. thiooxidans_ cells and elemental sulfur is probably required for oxidation to proceed and there is some controversy whether the intact sulfur moiety enters the cell (Vishniac and Santer, 1957). The pathway of sulfur oxidation is controversial. Glutathione or protein-bound thiol groups may be required. While it was once thought that thiosulfate is an intermediate, sulfite is now considered as a more likely intermediate. In any case, oxidation of sulfur involves multiple cleavage of the S_8 ring (Trudinger, 1969).

Three major pathways for oxidation of thiosulfate are considered as possibilities (Peck, 1962; Trudinger, 1969). The first involves an oxidative condensation to form tetrathionate and its subsequent oxidation via trithionate and sulfite to sulfate (tetrathionase pathway). The other two pathways involve initial cleavage of thiosulfate, alternatively to form sulfide and sulfite or elemental sulfur and sulfite. These cleavage products are subsequently oxidized; APS may or may not be an intermediate. These pathways are not necessarily mutually exclusive; T. thioparus may be capable of expressing both a thiosulfate-cleaving pathway and a tetrathionase pathway (Trudinger, 1969).

With regard to the distribution of the species of Thiobacillus, a number of factors are important; substrate, pH, oxidation-reduction potential, temperature, salinity, and various nutrients (Sokolova and Karavaiko, 1968). Generally, the genus may be divided into two major groups, the acidophiles and the neutrophiles. Oxidation-reduction potential is an important environmental parameter; while only T. denitrificans can develop in the total absence of oxygen, T. thioparus has been shown to be a microaerophile (Kuznetsov and Sokolova, 1960). Most of the thiobacilli are mesophilic but a thermophilic species has been reported and development of thiobacilli in hot springs has been observed (Egorova and Deryugina, 1963). Marine strains of most species of thiobacilli have been isolated (Tilton, Cobet, and Jones, 1967; Tilton, Stewart and Jones, 1967; Adair and Gundersen, 1969a and b); halphilic strains have also been reported (Keller, 1969).

As early as 1898, Egounov hypothesized, on the basis of laboratory experiments, that at an intermediate depth of a stratified water column a film of growth of sulfur bacteria would occur. At this depth limited amounts of both oxygen and sulfide would be located. The film itself would interfere with further upward transport of sulfide. Egounov was able to demonstrate such a film in cylinders of water, containing mud at the bottom. A similar phenomenon was reported by Suckow and Schwarz (1963).

Thiobacilli were detected in small numbers in the water column in the Black Sea (Kriss, 1963). No definite trends were seen; occasional positive tubes were observed from 1-2000 m. Thiobacilli were also detected in the sediments. Sorokin (1962) was also unable to confirm the presence of a thin film of thiobacilli in the Black Sea by his cultural studies, but he did detect layers of maximal dark CO_2-fixation ("chemosynthesis") near the top of the sulfide zone (190-250 m). Sorokin (1962 and 1964) found that this "chemosynthesis" was stimulated by the addition of thiosulfate and concluded that it was due to thiobacilli.

Anagnostidis and Overbeck (1966) observed maximal numbers of thiobacilli in the deeper portion of the hypolimnion of the Plussee, a lake in northern Germany which contains sulfide in its summer hypolimnion. Kuznetsov, et al., (1963) observed a similar phenomenon in the meromictic Lake Belovod, U.S.S.R.; highest numbers were observed immediately above the sediment surface. In the eutrophic Kuibyshev Resevoir, U.S.S.R., Sorokin observed that the activity of thiobacilli, as measured by thiosulfate oxidation, was high throughout the hypolimnion (Sokolova and Karavaiko, 1960).

Some heterotrophic organisms are also capable of oxidizing reduced sulfur compounds, apparently without utilizing the resultant energy. Sulfide oxidation to thiosulfate has been observed in mammalian tissues (Sörbo, 1960). Sulfide oxidation by Sphaerotilus, Pseudomonas, Micrococcus, Leucothrix, and various yeasts and molds has been observed (LaRiviere, 1966; Skerman, Dementjeva, and Skyring, 1957). Guittoneau (1927) observed that Bacillus subtilis, B. mesenteroides, B. megatherium, Escherichia coli, Proteus vulgaris, and Actinomyces griseus can oxidize elemental sulfur to thiosulfate. Penicillium glaucum was observed to produce a mixture of thiosulfate and sulfate and Sterigmatocystis nigra produces sulfate only. A sulfur-oxidizing Streptomyces was isolated by Wieringa (1966). A number of heterotrophic bacteria, such as Thiobacillus trautweinii and Pseudomonas aeruginosa are known to be capable of oxidation of thiosulfate to tetrathionate (Trudinger, 1969) and further oxidation of the tetrathionate to sulfate may also take place in certain organisms (Peck, 1962). Rhodanese, the enzyme catalyzing the cyanolysis of thiosulfate, has been found in animal tissues and in a number of heterotrophic bacteria; this enzyme may be active in both oxidative and reductive pathways (Trudinger, 1969). The oxidation of sulfite to sulfate may be non-enzymatically catalyzed by metal ions (Heinberg, et al., 1953) or it may be enzymatically oxidized in mammalian tissues (Fridovich and Handler, 1956; Peck, 1962). In addition, cysteine may be oxidized by such organisms as Proteus vulgaris to produce sulfate instead of sulfide (Singer and Kearney, 1955). The significance of heterotrophic oxidation of sulfur compounds in natural waters is unknown.

The photosynthetic sulfur bacteria are grouped in two families, Thiorhodaceae and Chlorobacteriaceae (Breed, et al., 1957).

The family Thiorhodaceae, or purple sulfur bacteria, consists of 13 genera, which are distinguished entirely by their morphology (Breed, et al., 1957). These genera include Thiosarcina, Thiopedia, Thiocapsa, Thiodictyon, Thiothece, Thiocystis, Lamprocystis, Amoebobacter, Thiopolycoccus, Thiospirillum, Rhabdomonas, Rhodothece, and Chromatium. Chromatium is the best known genus, many of the other genera have not been studied in pure culture. The Thiorhodaceae have colors ranging from bluish-violet, pale purple, brown, to deep red, due to the combined effect of carotenoids and bacteriochlorophyll a (Breed, et al., 1957). The latter pigment absorbs light at 350-400 mμ, at about 580 mμ, and at 800-890 mμ "in vivo" (Knodrat'eva, 1965).

The Chlorobacteriaceae (green sulfur bacteria) are divided into 6 genera, Chlorobium, Pelodictyon, Clathrochloris, Chlorobacterium, Chlorochromatium, and Cylindrogloea, distinguished by their morphology (Breed, et al., 1957). The latter three genera are found as symbionts with other organisms. Only Chlorobium has been studied in any detail. This organism possesses either bacteriochlorophyll c or d, which absorb light "in vivo" at about 450-460 mμ and at about 720-760 mμ

(Stanier and Smith, 1960).

The physiology of the photosynthetic sulfur bacteria has been reviewed by Kondrat'eva (1961 and 1965) and Larsen (1952 and 1953). They are generally characterized as photosynthetic organisms which are capable of utilizing hydrogen sulfide and other sulfur compounds as electron donors, instead of water (Van Niel, 1941). While purple sulfur bacteria apparently are able to deposit sulfur intracellularly, Chlorobium deposits sulfur extracellularly. The sulfur which is produced during sulfide oxidation may be subsequently oxidized to sulfate (Kondrat'eva, 1965). Some photosynthetic sulfur bacteria, as well as some of the Athiorhodaceae (purple non-sulfur bacteria, which typically utilize organic compounds as electron donors during photosynthesis) are also capable of oxidizing thiosulfate and polythionates (Larsen, 1953; Peck, 1962; Kondrat'eva, 1965).

The oxidation of sulfur compounds and the reduction of CO_2 are quite closely coupled, and close agreement with the following equations has been demonstrated (Larsen, 1952; Trüper and Schlegel, 1964; Trudinger, 1969):

$$CO_2 + 2\ H_2S \xrightarrow{\text{light}} (CH_2O) + H_2O + 2\ S^{\bullet}$$

$$3\ CO_2 + 2\ S^{\bullet} + 5\ H_2O \xrightarrow{\text{light}} 3\ (CH_2O) + 2\ H_2SO_4$$

$$2\ CO_2 + Na_2S_2O_3 + 3\ H_2O \xrightarrow{\text{light}} 2\ (CH_2O) + Na_2SO_4 + H_2SO_4.$$

In addition to the wavelength of light and the availability of substrate, van Niel (1931) suggested that pH and amount of H_2S are important environmental factors controlling the development of the various photosynthetic sulfur bacteria.

Massive development of photosynthetic sulfur bacteria in stratified aquatic environments has been observed by many authors. Most of the extensive literature regarding their distribution was reviewed by Kondrat'eva (1965) and Hutchinson (1957). This massive development or bloom may be green, pink, red, brown, or purple in color and it may be due to more than 10^7 cells/ml. *Chromatium*, *Lamprocystis*, *Thiopedia*, *Thodothece*, *Chlorobium*, *Chlorochromatium*, and *Clathrochloris* are typically the dominant genera in such blooms.

Dark CO_2 Fixation

The modification of Sorokin (1958) of the C^{14} methodology of Steeman-Nielsen (1952) has been used by a number of workers to estimate the amount of chemolithotrophic productivity in a water body. It has been assumed that any fixation of CO_2 in the dark is due to chemolithotrophic productivity (chemosynthesis) (Sorokin, 1958). Sorokin (1964b) emphasized that chemosynthesis is truly secondary productivity since the substrates for chemolithotrophic organisms, especially the reduced sulfur compounds, can usually be traced ultimately back to a photosynthetic reduction (primary productivity).

Kuznetsov (1958) observed chemosynthetic rates up to 1.1 mg $C/m^3/hr$, which represented up to 3.3% of the photosynthetic rate, in Lake Bieloye, near Moskow. In Lake Belovod, Lyalikova (1957) observed maximal chemosynthesis near the top of the sulfide zone (about 6 mg $C/m^3/hr$). Sorokin (1957b) observed chemosynthetic rates of 0.9-2.2 mg $C/m^3/hr$ in Rybinsk Resevoir during the winter whereas rates of up to 2.5 mg $C/m^3/hr$ during the summer were observed (Sorokin, 1958b). Rates were usually highest just above the sediment surface, and much higher rates (10.1-62.5 mg $C/m^3/hr$) were observed in the sediment itself (Sorokin, 1958a). Sorokin observed rates of chemosynthesis up to 582 mg $C/m^3/hr$ in the sediments of other lakes in the U.S.S.R. Sorokin (1959) was also able to demonstrate that chemosynthetic organisms participate directly in the aquatic food chain; he observed that various planktonic and benthic invertebrates consume and assimilate methane and hydrogen bacteria. Feeding of zooplankton on sulfur bacteria has been demonstrated by other investigators (Ruttner, 1966). Salmanov (1964) observed chemosynthetic rates up to 2.0 mg $C/m^3/hr$ near the bottom of Knibyshev Resevoir, U.S.S.R., during the summer.

Near the top of the sulfide zone of the Black Sea, Sorokin observed chemosynthetic rates of 0.17-0.38 mg $C/m^3/hr$ (Sorokin, 1962, 1964a, and 1965). His results indicated that up to one third of the total CO_2 fixed in the water column was due to chemolithotrophic activity.

Steeman-Nielsen (1960) noted that such chemosynthesis results must be regarded with caution, since dark CO_2 fixation actually represents the sum of chemolithotrophic and heterotrophic CO_2 fixation. Heterotrophic organisms have long been known to be capable of fixing small amounts of CO_2. The mechanisms of heterotrophic CO_2 fixation were reviewed by Wood and Stjernholm (1962).

A number of workers have investigated the role of heterotrophic organisms in the fixation of CO_2 in aquatic systems (Jones, et al., 1958; Sorokin, 1964; Romanenko, 1964a; Zharova, 1964). Romanenko reported that 2-8% of the total biomass of heterotrophic bacteria is due to fixation of CO_2. Seki (1967a) observed that dark assimilation of CO_2 in seawater is enhanced by the addition of organic matter. Heterotrophic CO_2 fixation by marine diatoms in the dark was also reported by Seki (1967b).

Some workers have applied a correction for heterotrophic uptake to their chemosynthetic results. This correction factor is based on the biomass and average generation time of the heterotrophic population (Sorokin, 1964).

Sorokin (1965) observed that heterotrophic CO_2 fixation was less than 3% of the dark fixation in the upper part of the sulfide zone of the Black Sea.

In order to avoid some of these difficulties, Romanenko (1964a and b) determined the "potential capacity" of heterotrophic and chemolithotrophic organisms to fix CO_2 in the dark. To samples of water or sediment, various organic and inorganic nutrients were added prior to incubation in the dark in the presence of labelled bicarbonate. He observed that the potential capacity of heterotrophic organisms greatly exceeded that of the chemolithotrophic organisms. A similar technique was used by Sorokin (1962) to estimate the potential capacity of the sulfur bacteria in the Black Sea. Unfortunately it is difficult to relate these results to natural substrate conditions.

Limnology of Oyster Pond

Oyster Pond lies adjacent to Vineyard Sound about two miles east of Woods Hole, Massachusetts. Because of its proximity to the scientific institutions in Woods Hole, this pond has been the subject of a considerable amount of investigation. This work was reviewed by Emery (1969).

The bathymetry and surrounding topography are shown in Fig. 1. It is about a kilometer long and is oriented in a North-South direction. The total area is 0.250 km^2. Most of the shoreline is relatively steep and forested while the shoreline of much of the southern part of the pond is shallower with few trees. There are a number of year-round and summer residences along the banks. The pond consists of three basins. The southern basin is the deepest (6.5 m). The middle basin is not a closed depression but is broad and flat with an average depth of 3.2 m. The northern basin has a maximum depth of 4.5 m. While the bottom is covered with boulders along much of the shoreline, most of the bottom in the basins is covered by as much as 6 m of sediment (Emery, 1969).

The steeper portion of the shoreline is composed of boulders, gravel, and coarse sand (Buzzards Bay recessional moraine). Much of the shallower sloping shoreline is an outwash plain consisting of sands and gravels. The basins of Oyster Pond are kettles, formed by the eventual melting of blocks of glacial ice which remained partly buried in unconsolidated deposits after the recession of the glacier (Emery, 1969).

The southern basin of Oyster Pond is separated from Vineyard Sound by a baymouth bar. The abundant oysters in the pond in colonial times accounted for the name of the pond. As early as 1767, the oyster fishery was declining, possibly a result of partial closure of the inlet from Vineyard Sound. This opening may have closed primarily through longshore drift of beach sand, but the construction of a railroad and a road along the shore in the 1870's completed the closure. The pond presently drains through a series of marsh ponds into Vineyard Sound; there are conduits through the road and railroad embankments (Emery, 1969).

There are no major surface tributaries but there is a net outflow from the pond of 1200-4000 m^3/day, while evaporation accounts for about 400 m^3/day. The majority of the freshwater entering the pond probably enters as ground water through shoreline springs, with minor contributions from septic tank effluent and direct precipitation on the pond. Some seawater enters the pond on especially high tides through the outlet and during severe storms in the form of spray (Emery, 1969).

Oyster Pond currents result mainly from wind-drag.

During periods of typical wind conditions, there is a counter-clockwise flow in the main part of the pond, while the currents in the northern basin flow in a clockwise direction (Emery, 1969).

Temperature and Cl‰ measurements of the northern and southern basins for the period October, 1963, through December, 1965, were reported by Emery (1969). In the northern basin a summer thermocline was present, while during the rest of the year the water column was nearly isothermal. No saline stratification was observed in the northern basin and an increasing trend in Cl‰ from about 1.0 ppt in October, 1963, to about 2.5 ppt in December, 1965, was noted. This increasing Cl‰ was also observed in the top 4 m of the southern basin. The southern basin exhibited saline stratification throughout year except immediately after the periods of complete circulation. Such overturns occurred in November, 1963, and March, 1964, but not again until April, 1967. During this period, the pond could be classified as meromictic (Hutchinson, 1957). A Cl‰ as high as 6 ppt was often observed near the bottom of the southern basin while the surface Cl‰ never exceeded 2.5 ppt. In addition to the halocline at about 4-5 m, there was a pronounced summer thermocline at about the same depth of the southern basin.

The ratios of the major ions of Oyster Pond water are similar to seawater indicating that the salt content is derived primarily from dilution of seawater (Emery, 1969). The bicarbonate content, though, is relatively high, probably a result of decomposition of organic matter. The deep water of the southern basin exhibited a slightly lower $SO_4^{=}:Cl^{-}$ ratio (0.1343) than expected from dilution of seawater (0.1394), suggesting the activity of sulfate-reducing bacteria. Emery noted that the salt content of the local ground water and tap water is quite low (Cl‰ = 0.036 ppt and 0.011 ppt, respectively).

Emery reported that the hypolimnion of the southern basin lacked dissolved oxygen and contained hydrogen sulfide throughout the year, except immediately after overturns. The upper portion of the hypolimnion had a milky appearance, presumably due to colloidal elemental sulfur. Hydrogen sulfide was observed in the hypolimnion of the northern basin during the summer months only. The highest pH occurred at about 1 m; this epilimnetic pH varied between 7 and 9. Walsh (1956b) observed a diurnal pH cycle, with highest pH occurring in the afternoon. An annual pH variation was observed by Walsh (1966), with highest pH during the summer. The hypolimnetic pH was more constant (6.9–7.4). Walsh (1966) observed that the oxidation-reduction potential stratification reflected the oxygen-sulfide stratification. The Eh of the epilimnion varied between +243 and +443 mV, while the Eh of the hypolimnion ranged between -9 and -127 mV except during periods of complete circulation.

The shallow areas of the pond are partly covered by the growth of the sessile plants, *Certaophyllum demersum*, *Potamogeton crispus*, and *P. foliosus*; *Zannichellia palustris* and *Xyris congdoni* may also be present (Emery, 1969). The blue green algae, *Microcystis marina*, *Anabena* sp., *Oscillatoria* sp., and *Merismopedia* sp. are the dominant phytoplankton with lesser growth of diatoms such as *Chaeotcerus* sp. (Walsh, 1965b). This Myxophycean plankton is characteristic of warm, eutrophic waters (Hutchinson, 1967).

Among the important fish are white perch, yellow perch, sunfish, mummichubs, eels, and herring. Other vertebrates include turtles, frogs, muskrats, otters, and many water birds, including ducks, geese, swans, gulls, loons, herons, etc. The zooplankton include copepods and possibly tintinnids. A number of benthic animals, such as amphipids, polychaetes, oligochaetes, turbellarians, hydroids, and gastropods, are found in the shallower portions (less than 2 m). A number of insects and their larvae are also present (Emery, 1969).

The only reports of bacteriological studies are rather limited. (Emery (1969) reported the results of coliform surveys, which indicated lower numbers of *Escherichia coli* after an open sewage drain was diverted. Walsh (1966) reported an intense bloom of *Chlorobium* near the top of the sulfide zone of the northern basin during the summer of 1969.

The high concentrations of dissolved organic matter in Oyster Pond are typical of eutrophic waters. Menzel observed total dissolved organic carbon concentrations of 5-10 ppm C in the water column during June, 1964, and May, 1965 (Emery, 1969).

Walsh (1965b) reported a diurnal cycle in dissolved carbohydrates. On 7/22-7/23/64, dissolved carbohydrate in inshore waters varied between 1.67 and 2.92 ppm (as sucrose) with highest values in the afternoon. Results of a second study on 7/31-8/1/64, in three locations were similar. Again, highest values were observed in the afternoon; the concentration varied between 1.57 and 3.54 ppm.

Walsh (1966) observed seasonal changes in the concentration of dissolved carbohydrate. Near the surface of the northern basin and at 2 m, the concentrations were lowest during the February plankton bloom and highest concentrations were observed later in the spring after the bloom. Concentrations of dissolved carbohydrate near the bottom of the northern basin were especially high (4.39 ppm) in July and August during the Chlorobium bloom. The annual cycles observed near the surface and at 3 m of the southern basin were similar to those observed at 0 and 2 m of the northern basin. At 6 m, concentrations were lowest in the spring and increased to 4.1-4.2 ppm in August and September.

The dissolved carbohydrate concentrations were considerably higher in Oyster Pond than in most other Cape Cod waters surveyed by Walsh (1965a).

Chlorophyll a concentration has been used as an index of phytoplankton biomass. Walsh (1966) observed an inverse relationship between the annual cycles in the concentration of dissolved carbohydrate and the chlorophyll a content of the plankton in the epilimnion of both basins. The chlorophyll content ranged between 2.68 and 30.92 ppb except during the Chlorobium bloom near the bottom of the northern basin during July and August. At that time, concentrations as high as 101 ppb were observed.

Productivity measurements were made by several different methods by Walsh, Yentsch, and Emery (Walsh, 1965a and b; Emery, 1969). The productivity was highest in the spring, lower in the summer, attaining a secondary peak near the end of the summer, and lowest in the winter. The relatively high productivity is consistent with the classification as a eutrophic pond. Emery (1969) attempted to construct an organic carbon budget for the pond, but the data were not sufficiently complete for this purpose.

Emery (1969) found that the floor of most of the pond was covered with a gray organic silt. At station 18 (Fig. 1) black organic silt was found. The sediments in the deeper portions had a smaller grain size than those in the shallower portions. Near the shores, the sediments graded into sands, and along the south shore the sands were especially coarse.

Carbonate, Kjeldahl nitrogen, and organic carbon content of the sediments were determined by Hülsemann (Emery, 1969). Calcium carbonate content ranged from 0.4-3.5% and averaged 1.0%. The organic carbon content was quite high, ranging up to 16.0%, except in the sands (0.3-2.5%). Kjeldahl nitrogen was also high, ranging up to 1.85%. The basin sediments had carbon : nitrogen ratios less than 10.0, while the ratio was higher in nearshore samples, where cellulose-containing debris from terrestrial vegetation was present. Walsh (1965a) found the total organic matter content at station 18 to be 19.15%.

Degens analyzed sediment samples from stations 3 and 18 for amino acids, amino sugars, diaminopimelic acid, and aminobutyric acid (Emery, 1969). The amino acid nitrogen accounted for 4.5-8.9% of the total Kjeldahl nitrogen of the sediments.

The C^{12}/C^{13} ratio of Oyster Pond sediments was determined by Sackett (Emery, 1969). For 8 samples, the δC^{13} ranged from -22.8 to -25.1 ppt, similar to that found in marine sediments. The methane in the sediments, on the other hand, was enriched in C^{12} (δC^{13} = -56 to -60 ppt), suggesting that fractionation may have occurred during bacterial methanogensis.

All of the sediments contained hydrogen sulfide. Bubbles of gas were observed to emanate from the organic silt sediments; this gas was found to consist largely of methane. Emery (1969) estimated the rate or escape of methane from the sediments to be about 0.0018 ml/g dry weight/day.

The coarse fractions of sediment samples were found by Emery (1969) to consist mostly of quartz grains, as well as feldspars, muscovite, some heavy minerals, blackened remains of parts of terrestrial plants, bits of hematite partly altered to limonite, cinders, and small grains of anthracite coal. The latter two constituents originated from the nearby railroad. The fine fraction was examined by Hathaway with x-ray diffraction methods (Emery, 1969). The 2-62 μ fraction consisted mainly of quartz and feldspar while the $< 2\ \mu$ fraction consisted largely of illite, kaolinite, and amorphous silica; much of this silica may have been the remains of diatom frustules. Each of the fractions contained pyrite (5% of the 2-62 μ fraction and 2% of the $< 2\ \mu$ fraction).

Stratigraphic evidence obtained by Deevey (1948) and Emery (1969) were used to construct a history of Oyster Pond. After the kettle basins were formed, they were filled with freshwater. This was about 11,770 ± 300 yr ago. As the sea level rose, the basins filled with seawater, first the southern basin, then the middle basin, and finally the northern basin. The interstitial water of sediments 700-1100 years old had a Cl‰ of 16 ppt, similar to that in the present Vineyard Sound. The Cl‰ then decreased, probably the result of the gradual building of a baymouth bar. About 70 years ago, the Cl‰ started to drop more rapidly; this was due to the construction of the railroad embankment. A number of sand layers were observed; these were felt to be the results of hurricanes washing sand into the pond. Emery estimated the current sedimentation rate to be about 340 cm/1000 yr.

MATERIALS AND METHODS

Sampling Locations

Most of the work was done at station 18, in the deepest part of the southern basin (6.5 m deep). Some work was also done in the northern basin at station 3 (4.5 m deep). Sampling locations are illustrated in Fig. 1.

Sampling Methods

A small row boat, kindly loaned by K. O. Emery, was used for most work. On one occasion samples were obtained through a hole in the winter ice. Sampling gear was lowered by hand, using light nylon line.

A "Coke bottle sampler" of the Meyer type (Ruttner, 1966) was used for most chemical work. This sampler has the advantage of simplicity and capability of sampling narrow stratifications. When large volumes of water were required, a three liter Van Dorn sampler was used. Generally, Cobet samplers (Jones, 1968) were utilized for bacteriological work. The capillaries and rubber bulbs were autoclaved at 121 C for 15 min and the samplers were assembled immediately after autoclaving with minimal exposure to air. On one occasion bacteriological samples were obtained with a Niskin sterile sampler (Niskin, 1962). A Van Veen type dredge was used to collect sediment samples.

Physical Factors

Water temperature. Water temperature was measured by plunging a thermometer into the "Coke bottle sampler" immediately after collection.

Transparency. Water transparency was determined with an eight inch white Secchi disc. The extinction coefficient, k, was calculated:

$$k = 1.7/D$$

where D is the depth at which the disc was no longer visible. The extinction coefficient can then be used to estimate the amount of light reaching any depth:

$$k = \frac{2.3(\log_{10} Id_1 - \log_{10} Id_2)}{d_2 - d_1}$$

where Id_1 is the intensity (in percentage of incident radiation) at d_1 meters and Id_2 is that at d_2 meters (Harvey, 1963).

Chemical Analyses of the Water

pH. A Beckman Model G pH meter equipped with small glass and calomel electrodes was used to measure the pH of freshly collected samples. The meter was standardized using pH 6.50 phosphate buffer (Radiometer).

Salinity. Salinity was measured by the Mohr titration (Strickland and Parsons, 1965). In this procedure, the precipitable halides in a 10 ml sample were titrated with 0.28 N $AgNO_3$ (Mallinckrodt Analytical), which had been standardized against I. A. P. O. "Copenhagen" water with a chlorinity of 19.369 ppt, using a chromate end point; 15 ml of 0.35% K_2CrO_4 (Mallinckrodt Analytical) was added prior to the start of the titration. The resultant chlorinity was converted to salinity by the Knudsen equation:

$$S‰ = 0.030 + 1.8050\ Cl‰ .$$

Dissolved Oxygen. The Winkler titration was used to measure the oxygen content (Strickland and Parsons, 1965). In this procedure, the dissolved oxygen is "pickled" by adding a divalent manganese solution and an alkaline iodide solution. Higher valence manganese hydroxides, in amount equivalent to the amount of oxygen present, are formed; the samples are now stable indefinitely. When acidified, the manganese is reduced to the divalent state and iodine is liberated. The iodine is titrated with thiosulfate using a starch indicator.

The following reagents were used:

A. 36.5% $MnSO_4 \cdot H_2O$ (Fisher Certified).

B. 50 g NaOH (Fisher Certified) in 50 ml water
50 g KI (Fisher Certified) in 50 ml water
The two solutions mixed.

C. 0.2% soluble starch (Baker).

D. 0.010 N $Na_2S_2O_3$ (0.10 N Fisher Certified Solution diluted 1:10).

The samples were introduced from the sampler into 60 ml glass-stoppered bottles with minimal agitation. Within an hour, 0.2 ml reagent A and 0.2 ml reagent B were introduced and the bottles were stoppered, entrapping no air bubbles. The bottles were thoroughly shaken and stored in the dark; 0.2 ml concentrated H_2SO_4 (Fisher Reagent) was added, dissolving the precipitate. Shortly after acidification, 50.0 ml were titrated with the thiosulfate to a pale straw color, at which point 5 ml of starch were added, and the titration was concluded rapidly (end point = colorless 20 seconds). The results were expressed in ppm O_2.

Sulfide. The colorimetric method of Packmayr (1960), as modified by Truper and Schlegel (1964), was used to measure sulfide. In this determination the sulfide is incorporated directly and stoichiometrically into methylene blue. Immediately upon collecting the sample, 1.0 - 10.0 ml, containing less than 90 μg sulfide, was added to 20 ml 2% zinc acetate (Baker Analyzed) in a 100 ml volumetric flask, thereby fixing the sulfide as ZnS. Since this salt is quite stable the assay may be completed up to two days later. The flask was filled to approximately 80 ml with water, followed by 10 ml of 0.2% N,N-dimethyl-p-phenylenediamine sulfate solution (Eastman) in 20% v/v H_2SO_4 (Fisher Reagent). The flask was swirled and 0.5 ml of 10% $FeNH_4(SO_4)_2$ solution (Mallinckrodt Analytical) in 2% H_2SO_4 (Fisher Reagent) was added immediately. The flask was filled to the mark, mixed, and allowed to stand 30 min before measuring at 670 mμ against a water blank in a 1 cm cuvette in a Beckman DU or Zeiss PMQ II spectrophotometer. The sulfide concentration was calculated by reference to a standard curve. The procedure was also checked by comparing with a gravimetric analysis using CdS; the two methods were found to agree within ± 5%.

Thiosulfate and Polythionates. A modification of the procedure of Sörbo (1957) was used to measure thiosulfate and polythionates. This method is based on the rapid conversion of thiosulfate to thiocyanate at room temperature in the presence of cupric ions. The thiocyanate forms a colored complex with ferric ions. The conversion of polythionates to thiocyanate is spontaneous but takes about 30 min for completion; thus, for the determination of polythionates, the copper reagent is omitted.

For analysis, a 30 ml sample was added in the field to a bottle containing 1.0 ml of 0.1 M $CdCl_2$ (Mallinckrodt Analytical), thus removing the sulfide as CdS. The sample was then filtered through a 0.45 μ membrane (Millipore) using a Swinnex unit (Millipore) attached to a 30 ml syringe. The filter was washed twice with 5 ml water and the volume of filtrate made to 50 ml with water. The filtrate was divided into four 12 ml portions, which were treated as follows:

1. Reagent Blank. Add 1.0 ml water and reagents in this order: 1.5 ml ferric nitrate solution (25 g $Fe(NO_3)_3 \cdot 9H_2O$ (Fisher Certified) + 50 ml Concentrated HNO_3 (Fisher Reagent) made to 250 ml with water); 1.5 ml of 0.1 M NaCN (Baker Analyzed); 1.0 ml of 1.0 M $CuCl_2$ (Fisher Certified). This measures only preformed thiocyanate.

2. Thiosulfate + Polythionates. Add 1.0 ml water and 1.5 ml NaCN. Wait 30 min and add 1.0 ml $CuCl_2$ and 1.5 ml $Fe(NO_3)_3$.

3. Polythionates Only (as Tetrathionate). Add 2.0 ml water and 1.5 ml NaCN. Wait 30 min and add 1.5 ml $Fe(NO_3)_3$.

4. Plus Internal Standard. Add 1.0 ml thiosulfate solution (12.0 meq/liter - 0.1 N Fisher Certified Solution diluted 12.0:100) and reagents as in 2.

The absorbance at 460 mμ was determined in 5 cm cuvettes against the reagent blank with a Zeiss PMQ II spectrophotometer. The method was found to be sensitive to 2 μeq thiosulfate/liter. The internal standard was necessary because sea water interferes with the reaction; the standard curve always was a straight line passing through zero, the slope merely decreased with increasing sea water concentration. The results were expressed in ppm S.

Sulfur. The presence of elemental sulfur was suggested by a milky turbidity of the water sample. Van Gemerden (1967) used the absorbance of ethanol solutions of sulfur at 260 mμ for determining the amount of sulfur in *Chromatium* cells. A similar method using hexane was reported by Maurice (1957).

This idea was adapted for use with water samples. It was found that benzene solutions of sulfur absorbed strongly at 280 mμ. In the concentration range useful for pond water, organic interference was negligible. In 1 cm cuvettes, the standard curve was linear at least as high as 40 ppm S.

Water samples, usually 50 ml, were filtered in the field through 0.45 μ membranes (Millipore) using a Swinnex unit (Millipore) attached to a syringe, and the filters were washed with distilled water. After drying, the membranes were extracted with benzene (Fisher Certified Spectranalyzed) in a Soxhlet extractor. Extraction times of 6 to 8 hours were found to be sufficient. The volume of the extract was made to 200 ml and the absorbance at 280 mμ was measured in a Zeiss PMQ II spectrophotometer using quartz cuvettes.

Sulfite. Sulfite was determined by the fuchsin-formaldehyde method of Pachmayr (1960) as modified by Trüper and Schlegel (1964). The sample was added to 20 ml of 2% zinc acetate (Baker Analyzed) in a 100 ml volumetric flask. The flask was then filled to approximately 80 ml with water, followed by the addition of 10 ml fuchsin solution (100 mg Bacto Basic Fuchsin in 250 ml 12.5% (v/v) H_2SO_4 (Fisher Reagent)). The flask was shaken and 10 or more min later 1.0 ml of 36.8% formaldehyde (Fisher Certified) was added. Exactly 10 min after the formalin was added, the absorbance at 570 mμ was measured in 1 cm cuvettes in a Zeiss PMQ II spectrophotometer. A standard curve was prepared with Na_2SO_3 (Mallinckrodt Analytical).

Sulfate. Sulfate was measured gravimetrically after Rieman, *et al.*, (1951). A 50 ml sample was mixed with 1.0 ml of 0.1 M $CdCl_2$ (Mallinckrodt Analytical) and then filtered through a 0.45 μ membrane (Millipore) using a Swinnex unit (Millipore) on a syringe. This removed the sulfide and any suspended material. The filter was washed with several rinses of distilled water. The filtrate was mixed with 30 ml of 10% $BaCl_2$ (Fisher Certified) in a 250 ml beaker and 0.75 ml concentrated HCl (Fisher Reagent) was added. The beaker was covered with a watch glass and was held in a boiling water bath for 4 hr. After allowing the beaker to remain at ambient temperature for 24 hr, the contents were filtered through a pre-combusted and tared Gooch crucible containing a glass fiber filter (Schleicher and Schuell). The filter was washed with distilled water, combusted at 500 C for 8 hr, cooled in a desiccator, and weighed.

Inorganic Phosphorus and Nitrogen. Phosphate, nitrate, nitrite, and ammonium were all determined on water samples which had been filtered through 0.45 μ membranes (Millipore); thus, this data includes only "soluble" forms. The samples were then frozen in Whirl-Pak Bags (Scientific Products) for storage prior to analysis; samples stored in this manner are probably stable for many weeks with regard to phosphate and inorganic nitrogen compounds (Strickland and Parsons, 1965). Due to a slight precipitate upon thawing (probably sulfur), samples were filtered through filter paper (Eaton-Dikeman) immediately prior to analysis.

Phosphate. The method of Murphy and Riley (1962), as described by Strickland and Parsons (1965), was used to measure reactive phosphorus, which is usually referred to as inorganic phosphate. In this method, a reagent containing ascorbic acid, molybdic acid, and trivalent antimony is used to form a stable blue-purple complex with the phosphate in the sample, the absorbance of which is measured colorimetrically.

The following reagents were used:

A. 3.0% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.

B. 140 ml concentrated H_2SO_4 (Fisher Reagent) plus 900 ml water.

C. 5.4% L-ascorbic acid (Fisher Reagent).

D. 0.34 g potassium antimonyl tartrate (Merck) in 250 ml water.

E. 100 ml A, 250 ml B, 100 ml C, plus 50 ml D mixed.

To 100 ml sample at ambient temperature, 10 ml reagent E were added. Between 5 min and 2 hr later, the absorbance at 885 mu was measured against distilled water in 5 cm cuvettes in a Zeiss PMQ II spectrophotometer. Absorbance of reagent blanks and standards, prepared with KH_2PO_4 (Fisher Certified) were also measured. The results were expressed as ppb P.

Nitrite. The procedure of Bendschneider and Robinson (1952), described by Strickland and Parsons (1965), was used to measure nitrite. In this procedure, the nitrite reacts with sulfanilamide under acidic conditions producing a diazo compound, which reacts with naphthylethylenediamine to form an azo dye, which is measured colorimetrically.

The reagents used were:

A. 1.0% sulfanilamide (Eastman) in 10% (v/v) HCl (Fisher Reagent).

B. 0.10% N-(1-Naphthyl)-ethylenediamine dihydrochloride (Fisher Reagent).

To a 50 ml sample, 1.0 ml reagent A was added and allowed to react for 2-8 min. Then, 1.0 ml reagent B was added and after 10 min - 2 hr, the absorbance at 543 mμ was measured against distilled water using 5 cm cuvettes in a Zeiss PMQ II spectrophotometer. Absorbances of reagent blanks and standards, prepared with $NaNO_2$ (Fisher Certified), were also measured. The results were expressed as ppb N.

Nitrate. Nitrate was determined by the method of Morris and Riley (1963), as modified by Strickland and Parsons (1965). The nitrate is reduced to nitrite by passage through a column of amalgamated cadmium filings. The resultant nitrite is then determined by the sulfanilamide-naphthylethylenediamine method.

The columns were prepared by joining 20 cm of 30 mm tubing on to 30 cm of 8 mm tubing. The opposite end of the smaller tubing was drawn out slightly to accommodate a short piece of rubber tubing. The flow rate was controlled with a screw clamp.

To prepare cadmium filings, sticks of cadmium metal (Fisher Certified) were filed with a coarse hand file. The 0.3-2.0 mm fraction was collected and about 100 g were stirred with 100 ml 1% $HgCl_2$ (Fisher Certified) for 3 min.

After decanting off the liquid, the amalgamated filings were washed several times with distilled water. The filings were poured into columns which had glass wool plugs in the bottom. Columns which were 20 cm long were prepared. When not in use the filings were covered with reagent B.

The following reagents were used:

A. 100 g NH_4Cl (Fisher Certified) in 500 ml water.

B. Reagent A, diluted 1:40.

C. 1.0% sulfanilamide (Eastman) in 10% (v/v) HCl (Fisher Reagent).

D. 0.10% N-(1-Naphthyl)-ethylenediamine dihydrochloride (Fisher Reagent).

To 80 ml sample, 2.0 ml reagent A were added; the sample was then added to a column. The flow rate was adjusted to about 6 ml per min. The first 25 ml were discarded, and 50 ml were collected in a graduated cylinder. To this, 1.0 ml reagent C was added and after 2-8 min, 1.0 ml reagent D was added. Between 10 min and 2 hr later, the absorbance at 543 mμ was measured against distilled water in 1 cm cuvettes in a Zeiss PMQ II spectrophotometer. Reagent blanks and standards prepared with KNO_3 (Fisher Certified) were also measured. The results were expressed as ppb N and a correction was made for nitrite initially present.

<u>Ammonium</u>. The method of Richards and Kletsch (1965), as modified by Strickland and Parsons (1965), was used to measure ammonium concentration. The ammonium is oxidized by

alkaline hypochlorite in the presence of a bromide catalyst. The excess hypochlorite is removed by arsenite, the solution acidified, and the resultant nitrite is determined by the sulfanilamide-naphthylethylenediamine method. The method suffers from interference by amino acids.

The following reagents were used:

A. 330 g NaOH (Fisher Certified) in 2000 ml water.

B. 1.5 ml 1.5 N NaOCl (Chlorox) in 100 ml reagent A.

C. 4.0% As_2O_3 in 6.0% NaOH (Fisher Certified).

D. 0.6% KBr (Baker Analyzed).

E. 0.55 g sulfanilamide (Eastman) in 272 ml 1:1 (v/v) HCl (Fisher Reagent):H_2O, water to 500 ml.

F. 0.1% N-(1-Naphthyl)-ethylenediamine dihydrochloride (Fisher Reagent).

All reagents, blanks, and standards were prepared using double distilled, deionized water. All glassware was freshly rinsed in 10% (v/v) HCl followed by distilled water rinses.

To 50 ml sample, 10.0 ml reagent B and 1.0 ml reagent D were added. After 3.5-4 hr at ambient temperature, 2.0 ml reagent C were added, followed, after 2 min, by 10.0 ml reagent E. After 3-8 min, 1.0 ml reagent F was added, and 10 min-2 hr later, the absorbance at 543 mu was measured against distilled water in 5 cm cuvettes in a Zeiss PMQ II spectrophotometer. Absorbances of reagent blanks and standards prepared with $(NH_4)_2SO_4$ (Mallinckrodt Analytical) were also measured. The nitrite initially present in the sample was subtracted from the results, which were expressed as ppb N.

Total Inorganic Carbon. CO_2 plus carbonates were determined by a procedure suggested by D. W. Menzel of Woods Hole Oceanographic Institution. Samples, in completely filled 60 ml glass-stoppered bottles, were kept on ice until 10 ml quantities could be sealed in glass ampoules. All manipulations were performed with minimal agitation. They were frozen until analysis, at which time they were thawed, opened, and 1.0 ml aliquots were taken. These were mixed with 0.5 ml 50% (v/v) HCl (Fisher Reagent) in the apparatus shown in Fig. 2. In this apparatus, the CO_2 liberated from the water sample by the acidification was carried by a flow of 200 ml/min of CO_2-free N_2 gas through a drying tube into a Beckman IR 215 infrared analyzer. The apparatus was a modification of one used by Menzel and Vaccaro (1964) for dissolved organic carbon. The standard curve was prepared by injecting known volumes of CO_2 gas into the system with a Hamilton syringe.

Analyses of Sediments

Dry Weight and Ash Weight. A gross estimate of water and organic matter content of sediments was made. About 5-10 g of wet sediment was added to a pre-combusted and tared crucible and weighed to determine wet weight. After drying at 105 C for 24 hr, the crucibles were weighed again, the weight loss was considered to be water. The crucibles were ashed in a muffle furnace at 600 C for 24 hr; the weight loss was considered to be combusted organic matter (Walsh, 1965a).

Chloride. Most of the water in a portion of the sediment was removed by suction. The salinity titration for water analysis was used for the determination of chloride in this interstitial water (Strickland and Parsons, 1965).

Water Soluble Sulfides. The methods used for the sulfide minerals were adapted from those of Kaplan, *et al.* (1963). The sediment sample, weighing approximately 50 g, was added to a tared 250 ml flask and weighed. The flask had been fitted with a stopper containing a dropping funnel and inlet and outlet tubes. A constant flow of nitrogen was maintained through the flask and the outlet was connected in series to two 200.0 ml 2.0% zinc acetate (Baker Analyzed) traps. A quantity of freshly boiled distilled water sufficient to cover the sample was added through the funnel. The flask was then heated in an 80 C waterbath until no more zinc sulfide precipitated; 2-3 hr was usually sufficient. The sulfide in the traps was determined by the colorimetric procedure used for water analysis; the free or water soluble sulfide was then calculated.

Acid Soluble Sulfides. Fresh zinc acetate traps were added to the above apparatus. Enough concentrated HCl (Fisher Reagent) to give an approximate 2N solution was added through the funnel, the flask was heated as above, and the traps analyzed for sulfide.

<u>Sulfate</u>. The flask contents were filtered through Schleicher and Schuell #576 filter paper and washed with hot, freshly boiled distilled water. Due to a slight turbidity, the filtrate was re-filtered through a 0.45 μ membrane filter (Millipore). The volume of filtrate and washes was measured, and the sulfate was measured gravimetrically (Rieman, <u>et al</u>., 1951).

<u>Sulfur</u>. The material on the above filters was dried at 105 C overnight and placed in an extraction thimble in a Soxhlet extractor. The procedure was an adaptation of that used by Berner (1964a). The sample was extracted with acetone (Fisher Certified) in the presence of approximately 0.5 g fine granular copper metal (Mallinckrodt Analytical) contained in the collection vessel. With the acetone acting as both the solvent and a catalyst, fresh extract is continuously supplied to the copper surface where immediate reaction with elemental sulfur takes place to form CuS. Extraction was continued for 2-3 hr which was found to be sufficient for essentially complete extraction of added sulfur. After the extraction was completed, the extract was evaporated almost to dryness and the flask was connected to the gas delivery system described above. With a constant flow of nitrogen through the flask, enough 2N HCl was added to completely cover the residue. The flask was heated as above and the evolved sulfide was collected in zinc acetate traps; the sulfide was then determined colorimetrically. This method is supposedly specific for elemental sulfur

in the presence of a variety of organic sulfur compounds.

Pyrite. The procedure of Kaplan, et al. (1963) was modified for determination of pyrite sulfur. All of the following treatments were performed on the contents of the extraction thimble in the Soxhlet extractor; reagents were added by decanting or pipetting into the top of the extractor. Any remaining organic sulfur was hydrolyzed successively with 100 ml portions of 6N HCl (Fisher Reagent) and 12N NaOH (Fisher Certified). After each of these hydrolysis steps, the sediment was washed with 50 ml water. It was then assumed that the remaining sulfur was in the form of pyrite.

This was oxidized by treatment with 200 ml aqua regia, HCl (Fisher Reagent): HNO_3 (Dupont Technical = 3:1 (v/v) . The residue was then extracted with 300 ml boiling water. The combined extracts were allowed to stand overnight and filtered through a 0.45 μ membrane filter (Millipore) to remove any subsequent precipitate. The sulfate in the filtrate was then determined gravimetrically.

Bacteriological Methods

With few exceptions media were incubated at ambient temperature (22-30 C). Whenever a shaker was employed, a rotary shaker at 200-250 rpm was used. Dilution blanks invariably consisted of 10% synthetic seawater (Lyman and Fleming, 1940). Media were sterilized by autoclaving at 121 C for 15 min; solutions of phosphates and bicarbonate were separately autoclaved at 10X concentrations; solutions of iron, manganese, and sulfide were filter sterilized with

0.22 μ membrane filters (Millipore). When direct microscopic counts were performed, a Petroff-Hausser counting chamber was employed. All microscopic work was done with a Zeiss WL Research Microscope equipped with both phase-contrast and bright field optics.

Enumeration of Thiobacilli. Various plating procedures for the enumeration of thiobacilli were attempted but they did not prove to be specific enough for use with mixed inocula. A tube dilution procedure was utilized instead. Five replicate tubes with 1.0 ml inocula were employed at each dilution. In order to have the final concentrations of the media constituents equal those given below after the addition of the inoculum, media were prepared somewhat more concentrated; that is, if 1.0 ml was to be inoculated into 10 ml medium, 9 ml of a 10/9 concentrate of the medium were used; if 1.0 ml was to be inoculated into 5 ml medium, 4 ml of a 5/4 concentrate of the medium were used. Most probable numbers (MPN) were evaluated using the tables in the *Standard Methods for the Examination of Water and Waste Water* (American Public Health Association, 1965).

Pure cultures of *Thiobacillus thioparus*, *T. thiooxidans*, and *T. denitrificans* served as positive controls and uninoculated tubes were included as well.

For *T. thioparus*, the data for 1968 were obtained using 10 ml tubes of the following medium:

Medium A

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0 mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0 mg
NH_4Cl	(Fisher Certified)	0.6 g
K_2HPO_4	(Fisher Certified)	2.0 g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	5.0 g
Synthetic Seawater	(Lyman and Fleming, 1940)	100 ml
Distilled Water		900 ml
pH		7.8

Tubes were incubated at ambient temperature for 3 weeks and were observed for turbidity or pellicle, drop of pH, and oxidation of thiosulfate. Thiosulfate oxidation was checked by titration with iodine; to 1.0 ml culture, 0.5 of ml 0.2% soluble starch (Baker Analyzed) was added, followed by titration to the first permanent blue color with 0.01 N iodine solution (Mallinckrodt Analytical).

The *T. thioparus* data for 1969 were derived using 5 ml tubes of the following medium, which is an adaptation of that of Vishniac and Santer (1957):

Medium B

brom thymol blue	(Difco)	0.05 g
NH_4Cl	(Fisher Certified)	0.4 g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.8 g
KH_2PO_4	(Fisher Certified)	4.0 g
K_2HPO_4	(Fisher Certified)	4.0 g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	10.0 g
Trace Metal Solution		10.0 ml
Distilled Water		990 ml
pH		7.8

Trace Metal Solution

E.D.T.A.	(Fisher Certified)	50.0	g
$ZnSO_4 \cdot 7H_2O$	(Fisher Certified)	22.0	g
$CaCl_2$	(Fisher Certified)	5.54	g
$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	5.06	g
$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	4.99	g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	(Fisher Certified)	1.10	g
$CuSO_4 \cdot 5H_2O$	(Fisher Certified)	1.57	g
$CoCl_2 \cdot 6H_2O$	(Fisher Certified)	1.61	g
Distilled water		1000	ml

pH adjusted to 6.0 with KOH (Fisher Certified)

The presence of the brom thymol blue in this medium provided a quick way to screen for organisms lowering the pH. Tubes were incubated on a rotary shaker for 3 weeks at ambient temperature. Tubes were observed for turbidity, drop of pH, and oxidation of thiosulfate.

The MPN was also compared with colony counts on spread plates of the following medium:

Medium C

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0	mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0	mg
brom thymol blue	(Difco)	0.05	g
$CaCl_2$	(Fisher Certified)	0.2	g
NH_4Cl	(Fisher Certified)	0.3	g
$MgCl_2 \cdot 6H_2O$	(Fisher Certified)	0.5	g
KH_2PO_4	(Fisher Certified)	1.35	g
K_2HPO_4	(Fisher Certified)	1.65	g

$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	5.0	g
Oxoid Ionagar #2		10.0	g
Distilled Water		1000	ml
pH		7.2	

Thiobacillus thioparus colonies on this medium were vivid yellow. A number of colonies from Medium C were inoculated into Medium B and Nutrient Broth (Difco).

In 1968 *T. thiooxidans* was enumerated in 10 ml tubes of the following medium:

Medium D

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0	mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0	mg
NH_4Cl	(Fisher Certified)	0.6	g
KH_2PO_4	(Fisher Certified)	2.0	g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	5.0	g
Synthetic Seawater	(Lyman and Fleming,1940)	100	ml
Distilled Water		900	ml
pH		4.8	

Tubes were incubated for 3 weeks at ambient temperature and were observed for turbidity or pellicle, drop of pH, and oxidation of thiosulfate.

Higher counts were obtainable with the following modification of Starkey's medium (1925). Therefore, it was used during 1969. Five ml tubes were sterilized by flowing steam at 100 C for 30 min.

Medium E

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0 mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0 mg
brom phenol blue	(Fisher Certified)	0.05 g
$CaCl_2$	(Fisher Certified)	0.2 g
NH_4Cl	(Fisher Certified)	0.3 g
$MgCl_2 \cdot 6H_2O$	(Fisher Certified)	0.5 g
KH_2PO_4	(Fisher Certified)	3.0 g
Distilled Water		1000 ml
pH		4.5

Sublimed sulfur (Fisher) was added separately to each tube (0.05 g). Tubes were incubated on a rotary shaker at ambient temperature for 3 weeks and were observed for turbidity, wetting of sulfur, and drop of pH.

Medium E, with 5.0 g/liter $Na_2S_2O_3 \cdot 5H_2O$ (Fisher Certified) substituted for the sulfur, was called Medium F.

T. denitrificans was enumerated during 1968 using 10 ml tubes of the following medium. Tubes of this medium contained Durham tubes in order to observe the production of gas.

Medium G

$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.01 g
NH_4Cl	(Fisher Certified)	0.5 g
$NaHCO_3$	(Fisher Certified)	1.0 g
KNO_3	(Fisher Certified)	2.0 g
KH_2PO_4	(Fisher Certified)	2.0 g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	5.0 g

Synthetic Sea Water	(Lyman and Fleming, 1940)	100 ml
Distilled Water		900 ml
pH		7.0

After 3 weeks of incubation at ambient temperature in an atmosphere of 90% N_2, 10% CO_2, tubes were observed for drop of pH, turbidity, oxidation of thiosulfate, and gas production.

During 1969, 5 ml tubes of the following medium were used instead of Medium G. Both media (G and H) are modifications of that of Baalsrud and Baalsrud (1954).

Medium H

$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.01 g
brom thymol blue	(Difco)	0.05 g
NH_4Cl	(Fisher Certified)	0.5 g
$NaHCO_3$	(Fisher Certified)	1.0 g
$MgCl_2 \cdot 6H_2O$	(Fisher Certified)	2.0 g
KNO_3	(Fisher Certified)	2.0 g
KH_2PO_4	(Fisher Certified)	2.0 g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	5.0 g
Distilled Water		1000 ml
pH		7.0

Tubes were incubated in an atmosphere of 90% N_2, 10% CO_2 at ambient temperature for 3 weeks. They were then observed for turbidity, drop of pH, oxidation of thiosulfate, and gas production.

The media and methods for the various thiobacilli used in 1968 and 1969 were compared on a number of occasions.

On 3/27/69 and 5/20/69, water samples from 5.0 and 6.5 m were supplemented with 0, 10^2, and 10^4/100 ml of T. thioparus, T. thiooxidans, and T. denitrificans. The inoculum of T. thioparus consisted of a four-day slant of strain #MS-5 on Medium C (without pH indicator). The T. thiooxidans inoculum was a four-day slant on Medium F (plus 10.0 g/liter Oxoid Ionagar #2, without pH indicator) of strain #MS-11. The inoculum of T. denitrificans was a six-day stab in Medium H (without pH indicator) of strain #C. The organisms were resuspended in 10% synthetic seawater (Lyman and Fleming, 1940) and the cell count determined by direct microscopic count. On 11/7/69 the two most probable number procedures for T. thioparus were compared with colony counts on spread plates of Medium C. Water samples from 5.5 and 6.2 m were supplemented with 0, 10^2, 10^3, 10^4, 10^5, 10^6 cells/100 ml of a four-day culture of strain #MS-5 in Medium B.

Pure Cultures of Thiobacilli. Colonial isolates of thiobacilli were obtained from most probable number tubes by streak plating on agar media of the same composition. Plates for T. thioparus and T. thiooxidans were incubated in 90% N_2, 10% CO_2. Pure cultures were obtained by two successive subcultures from single colonies. Contamination was also checked microscopically and by inoculating into tubes of Nutrient Broth (Difco) and Medium R (without agar) which is described below.

The origin of type cultures and Oyster Pond isolates of thiobacilli is described below:

T. thioparus	(#A) supplied by J. M. Shively, originally isolated by R. L. Starkey
"	(#MS-5) Oyster Pond (7/29/68, 5.0 m)
T. thiooxidans	(#B) A.T.C.C. #8085
"	(#MS-11) Oyster Pond (7/29/68, 5.0 m)
T. denitrificans	(#C) A.T.C.C. #23644
"	(#MS-17) Oyster Pond (9/8/68, 5.6 m)
Thiobacillus sp.	(#MS-25 Oyster Pond (8/14/69, 4.0 m)

The taxonomy of the various isolates was determined by comparing them with type cultures. For these comparative studies, the following techniques were used:

Gram stain: Hucker modification

Cell morphology and motility: Phase contrast - wet mounts

Colonial morphology: (7 days)

T. thioparus - Medium C (without pH indicator)

T. thiooxidans - Medium F (plus 10.0 g/liter Oxoid Ionagar #2, without pH indicator)

T. denitrificans - Medium H (without pH indicator) in air and in 90% N_2, 10% CO_2

Temperature relationships: Colonial growth on above solid media observed at 4, 20, 28, 37, and 55 C for 3 weeks.

pH relationships: Colonial growth on solid media at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 observed for 3 weeks

Anaerobic growth and gas production: Tubes of liquid media containing Durham tubes - air vs 90% N_2, 10% CO_2 (2 weeks) *T. thioparus* and *T. denitrificans* - Medium H

T. thiooxidans - Medium H at pH 4.5

Growth on thiosulfate: various liquid media

Growth on elemental sulfur: various liquid media

Final pH: (2 weeks)

T. thioparus - Medium H

T. denitrificans - Medium H; 90% N_2, 10% CO_2

T. thiooxidans - Medium H at pH 4.5 vs same medium with 10.0 g/liter sulfur substituted for thiosulfate

Growth in organic media: Nutrient Broth (Difco) and Medium R below

Rating of growth (colony size on agar media; turbidity and drop of pH in liquid media)

++++ luxuriant growth

+++ good growth

++ fair growth

+ slight growth

± questionable growth

0 no growth

Stock cultures of *T. thioparus* were maintained on slants of Medium C (without pH indicator). *T. thiooxidans* was maintained on slants of Medium F (plus 10.0 g/liter Oxoid Ionagar #2, without pH indicator). Stock cultures of *T. denitrificans* were maintained in stabs of Medium H (without pH indicator). Cultures were grown at ambient temperature for 4 to 6 days and then were stored for 2 weeks at 4 C before transferring.

Culture of Heterotrophic Thiosulfate Oxidizing Organisms. A number of heterotrophic thiosulfate oxidizing microorganisms were isolated from MPN tubes for thiobacilli in which the pH had risen substantially during thiosulfate oxidation. Culture was attempted using the media for the autotrophic thiobacilli as well.

Modifications of Starkey's medium (1935) for *T. trautweinii* were used for these organisms; the basic medium is as follows:

Medium I

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0	mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0	mg
NH_4Cl	(Fisher Certified)	0.6	g
KH_2PO_4	(Fisher Certified)	4.0	g
K_2HPO_4	(Fisher Certified)	4.0	g
$Na_2S_2O_3 \cdot 5H_2O$	(Fisher Certified)	10.0	g
Synthetic Seawater	(Lyman and Fleming, 1940)	100	ml
Distilled Water		900	ml
pH		6.6	

For subculture from MPN tubes, the medium was supplemented with either: A. 0.1 g/liter DL-asparagine (Eastman); B. 0.1 g/liter Bacto-peptone; C. 0.1 g/liter glucose (Difco Certified); or D. 0.1 g/liter yeast extract (Fisher). For plating, the medium was supplemented with 15.0 g/liter Bacto-agar plus 1.0 g/liter Bacto-peptone.

In an experiment to determine the effects of organic matter and thiosulfate on isolates, the medium was used with the following additions and deletions: E. plus 0.2 g/liter Bacto-peptone, without thiosulfate; F. plus 0.2 g/liter Bacto-peptone; and G. no additions or deletions. Cultures were followed with pH measurement, thiosulfate titrations, and direct microscopic counts.

On two occasions, a number of colonies were picked from nutrient agar plates in a random manner. These were inoculated into tubes of Medium I (plus 1.0 g/liter Bacto-peptone). After one week at ambient temperature, tubes were checked for growth, thiosulfate oxidation, and pH change.

Enrichment Cultures for Other "Colorless Sulfur Bacteria". On 1/15/70 samples from 3.0 and 6.5 m were obtained through the ice using a Niskin Sterile Sampler. Enrichments were set up in the following manner:

A. Unaltered water sample - 2000 ml - 4 C

B. Unaltered water sample - 2000 ml - 20 C

C. Air removed by bubbling N_2 for 2.5 hr, followed by 3 min with CO_2 - 2000 ml - 20 C

D. *Beggiatoa* enrichment technique of Faust and Wolfe (1961). Dried straw was extracted with boiling water eight times and then dried, and 5.0 g was then added to 2000 ml flasks and autoclaved. The water sample (2000 ml) was added and the flask incubated at 20 C.

E. *Thiovulum* enrichment technique suggested by C. Wirsen, Woods Hole Oceanographic Institution; modified from techniques of LaRiviere (1963). In this method a constant source of sulfide is provided; this consists of 10 mM $Na_2S \cdot 9H_2O$ (Fisher Certified) contained in a length of 10 mm tubing fitted with a sterile dialysis membrane, extending to the bottom of a 4000 ml flask. The flask contained 2000 ml 10% synthetic seawater which was pre-equilibrated with the sulfide source for 24 hr; the liquid in the flask then contained 6 to 7 ppm sulfide. The water sample (2000 ml) was then added. There was also a constant supply of air; this localized source of aeration was adapted from that of LaRiviere (1963). It consisted of a capillary pipette with a glass tube sealed around its drawn out end. The outer tube extended about 2 to 3 cm further into the water than the tip of the pipette; it also had a vent hole above the water line. Filter sterilized air was then bubbled very slowly into the medium. The sulfide source was replenished every 3 to 4 days and the dialysis membrane aseptically changed at weekly intervals.

The enrichment cultures were continued for 8 weeks and the pH, oxygen, and sulfide were periodically checked. The MPN of _T. thioparus_ was also determined on occasion. The bulk of the examination was microscopic; routinely, wet mounts of water, precipitated material on the bottoms of flasks, and flocs of growth were examined using phase-contrast. In addition to the gram stain, organisms in the enrichments were stained for volutin and lipoidal inclusions as indicated, and inclusions were pyridine-extracted to remove elemental sulfur.

The method of Scotten and Stokes (1962) was used to stain volutin. Dried films were fixed by immersing in methanol for 3 min, washed with water, and stained with 1.0% Bacto-methylene blue for 2 min. The slides were washed with water and wet mounts were prepared and sealed with paraffin. Volutin stains deep blue with this procedure.

Lipoidal inclusions were stained with 0.3% Sudan black B (Harleco) in 70% ethanol according to the method of Scotten and Stokes (1962). Equal amounts of stain and liquid material from enrichments were mixed on a slide, wet mounts prepared, and the slides sealed with paraffin. Burdon's method, which consists of staining dried films with the above Sudan black B solution, washing with xylene, and counterstaining with 0.5% safranin (Difco), was also employed. In either case, lipoidal inclusions stain very dark blue.

To remove inclusions of elemental sulfur, specimens were mounted under cover slips and pyridine was drawn through by placing a drop on one side and a piece of filter paper on the other (Skerman, _et al._, 1957). The extraction was observed microscopically.

Cultural and Optical Techniques for Photosynthetic Sulfur Bacteria. Liquid enrichment cultures using duplicate 1.0 ml inocula were used to demonstrate the presence of photosynthetic sulfur bacteria.

The medium of Larsen (1953) was used for *Chlorobium*. The medium was prepared just before use. Sixty ml glass stoppered bottles were used and the bottles were filled completely to exclude air.

Medium J

$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	0.5 mg
$MgCl_2 \cdot 6H_2O$	(Fisher Certified)	1.0 g
NH_4Cl	(Fisher Certified)	1.0 g
KH_2PO_4	(Fisher Certified)	1.0 g
$Na_2S \cdot 9H_2O$	(Fisher Certified)	1.0 g
NaCl	(Fisher Certified)	1.0 g
$NaHCO_3$	(Fisher Certified)	2.0 g
Distilled Water		1000 ml
pH		7.3

Bottles were incubated at ambient temperature six inches from a 50-watt incandescent bulb for 3 weeks. Due to the fact that purple sulfur bacteria developed in this medium as well, the bottles were checked often for growth.

Pure cultures were isolated using the same medium with the addition of 10.0 g/liter Oxoid Ionagar #2. A series of 1:10 dilutions from positive enrichments were added to tubes to which the medium was added. After the agar hardened, it was overlaid with a plug of the same agar medium. Tubes were incubated at ambient temperature about six inches from a 50-watt incandescent bulb until colonies appeared.

Similar techniques were used for the purple sulfur bacteria. The medium was adapted from the one described by Skerman (1967) for culture of <u>Chromatium</u> and had the following composition:

Medium K

$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	0.5 mg
NH_4Cl	(Fisher Certified)	0.1 g
KH_2PO_4	(Fisher Certified)	0.1 g
NaCl	(Fisher Certified)	0.1 g
$Na_2S \cdot 9H_2O$	(Howe and French)	0.1 g
$NaHCO_3$	(Fisher Certified)	2.0 g
Distilled Water		1000 ml
pH		7.8

Preparation and incubation were similar to the methods used for <u>Chlorobium</u>, and the above medium, with the addition of 10.0 g/liter Oxoid Ionagar #2 was used for isolation.

The unique absorption spectra of the photosynthetic bacteria was used as a tool for relative quantitative data for water samples from the pond and for characterization of cultures. The spectrophotometers employed were a Zeiss PMQ II and a Bausch and Lomb Spectronic 600.

"In vivo" spectra were obtained by adding the culture or pond water to cuvettes. "In vitro" absorption spectra were performed using methanol extracts. The method, essentially that of van Gemerden (1967), consisted of filtering water samples (50 ml was usually used) or cultures through 0.3 μ glass fiber filters (Gelman type A) in semidarkness and washing with distilled water . The filters were then extracted with 10 ml methanol (Fisher Certified) and the spectra measured using one cm cuvettes. For water samples, the wave lengths 660 mμ and 770 mμ were used for relative indexes of abundance of green and purple sulfur bacteria, respectively, as recommended by van Gemerden.

<u>Enrichment Cultures for Other Chemolithotrophic Bacteria</u>. Liquid enrichment cultures using triplicate 1.0 ml inocula were used to demonstrate the presence of other chemolithotrophic bacteria. So that the final concentrations of the media constituents would equal those given below, 1.0 ml inocula were added to 9 ml of 10/9 concentrates of the following media.

The medium of Harrington and Kallio (1960) was used for Methanomonas-like organisms:

Medium L

$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.05 g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.2 g
$(NH_4)H_2PO_4$	(Baker Analyzed)	0.8 g
K_2HPO_4	(Fisher Certified)	1.5 g
methanol	(Fisher Certified)	10.0 ml
Distilled Water		990 ml
pH		7.0

Tubes were incubated at ambient temperature for 3 weeks and were observed for pellicle or turbidity. Uninoculated tubes and tubes without methanol were included as controls.

The medium of Schatz and Bovell (1952) was used to cultivate Hydrogenomonas:

Medium M

$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.01 g
$CaCl_2 \cdot 2H_2O$	(Fisher Certified)	0.01 g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.2 g
$NaHCO_3$	(Fisher Certified)	0.5 g
KH_2PO_4	(Fisher Certified)	1.0 g
NH_4NO_3	(Baker Analyzed)	1.0 g
Distilled Water		1000 ml
pH		7.0

Tubes were incubated at ambient temperature in an atmosphere of 10% CO_2, 30% air, and 60% H_2 for 3 weeks and observed for turbidity. Uninoculated tubes and tubes incubated in air served as controls.

Wolfe's medium (1958) was used for Gallionella. Sterile FeS precipitate, prepared by the method of Kucera and Wolfe (1957) from equimolar quantities of $Na_2S \cdot 9H_2O$ (Fisher Certified) and $Fe(NH_4)_2SO_4 \cdot 6H_2O$ (Mallinckrodt Analytical), was mixed with an equal volume of sterile 2.0% Oxoid Ionagar #2. Two ml of this mixture were added to sterile screw cap tubes and slanted. After solidification, the following sterile salts medium was added:

Medium N

$CaCl_2$	(Fisher Certified)	0.1	g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.4	g
K_2HPO_4	(Fisher Certified)	0.5	g
NH_4Cl	(Fisher Certified)	0.1	g
Distilled Water		900	ml

Tubes bubbled with filter sterilized CO_2;
this yielded a pH of about 6.5

Tubes were incubated at ambient temperature with their caps tightly closed for 3 weeks. They were observed for the presence of the characteristic "growth ring" and the presence of *Gallionella* was confirmed microscopically. Uninoculated control tubes were included as well.

The medium of Leathen, *et al*. (1956) was used for *Ferrobacillus*:

Medium O

$Ca(NO_3)$	(Fisher Certified)	0.01	g
KCl	(Fisher Certified)	0.05	g
K_2HPO_4	(Fisher Certified)	0.05	g

$(NH_4)_2SO_4$	(Mallinckrodt Analytical)	0.15	g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.5	g
$FeSO_4 \cdot 7H_2O$	(Fisher Certified)	1.0	g
Distilled Water		1000	ml
pH		4.5	

Tubes were incubated at ambient temperature for 3 weeks and observed for oxidation of iron visually and using a permanganate titration:

Standard Permanganate: 3.2 g $KMnO_4$ (Mallinckrodt Analytical) per liter. Boil 10 to 15 min, let stand overnight, and filter through filter paper (Eaton-Dikeman). Standardize with 0.1 N $Na_2C_2O_4$ (Mallinckrodt Analytical).

Titration: 5.0 ml culture filtered with filter paper (Eaton-Dikeman) + 10 ml 50% (v/v) H_2SO_4 (Fisher Reagent) + 10 ml saturated $HgCl_2$ (Fisher Certified) + 20 ml $MnSO_4$ solution (51 g $MnSO_4 \cdot H_2O$ (Mallinckrodt Analytical) per liter of 13.8% (v/v) H_3PO_4 (Fisher Certified), 13.0% (v/v) H_2SO_4 (Fisher Reagent)). Mixture titrated with $KMnO_4$ until a permanent pink color is evident.

Uninoculated control tubes were included.

Media described by Skerman (1967) were used for the cultivation of nitrite and ammonium oxidizing nitrifying bacteria:

Medium P

$NaMoO_4 \cdot 2H_2O$	(Fisher Certified)	1.0	mg
$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	2.0	mg
$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.05	g
$CaCl_2$	(Fisher Certified)	0.02	g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	0.2	g
K_2HPO_4	(Fisher Certified)	1.0	g
NH_4Cl	(Fisher Certified)	1.5	g
Distilled Water		1000	ml
pH		8.5	

Medium Q

$NaNO_2$	(Fisher Certified)	3.0	g

substituted for NH_4Cl in Medium P.

Tubes were incubated at ambient temperature on a rotary shaker for 3 weeks and were observed for turbidity and the appearance of nitrite in Medium P and nitrate in Medium Q. The following spot tests described by Pramer and Schmidt (1965) were used to test for these end products:

Nitrite: 0.5 ml sample + 3 drops solution A (1.0% sulfanilic acid (Fisher Certified) in 30% (v/v) glacial acetic acid (Fisher Reagent)) + 3 drops solution B (0.3% alpha naphthylamine (Fisher Reagent) in 30% (v/v) glacial acetic acid (Fisher Reagent)). A red color indicates the presence of nitrite.

Nitrate: Destroy any nitrite present by boiling 5 ml sample + 3 ml solution A for 3 min or longer. Perform the test as for nitrite; there should be no color. Then add app. 1-5 mg acid-washed zinc dust (Fisher Certified). The development of a red color indicates the presence of nitrate.

Uninoculated tubes and tubes without nitrite or ammonium served as controls.

Enumeration of Heterotrophic Bacteria. Aerobic heterotrophic bacteria were enumerated by spread plating 0.1 ml inocula on Nutrient Agar (Difco) plates. On one occasion (7/29/68), the following medium was used as well:

Medium R

Bacto-peptone		1.0	g
Yeast extract	(Fisher)	1.0	g
Bacto-agar		15	g
Synthetic Seawater	(Lyman and Fleming, 1940)	750	ml
Distilled Water		250	ml
pH		7.5	

Usually, duplicate plates at each dilution were employed. Plates were incubated at ambient temperature for one week before counting macro colonies.

Anaerobic heterotrophic bacteria were enumerated by spread plating on Nutrient Agar (Difco) plus 0.5 g/liter sodium thioglycollate (Baltimore Biological Laboratory). Plates were incubated in an atmosphere of 90% N_2, 10% CO_2 at ambient temperature for one week.

Anaerobic heterotrophic bacteria were also enumerated in deep agar tubes of the same medium. These were prepared by mixing 0.1 ml inocula with 5 ml of the melted medium (45 C) in 13 x 100 mm tubes. After this medium had solidified an additional 2 cm overlay of the same medium was added. Tubes were incubated at ambient temperature for one week before colonies were counted.

Enumeration of Sulfide-Producing Bacteria. Deep agar tubes were also employed for the enumeration of dissimilatory sulfate-reducing bacteria. The medium was that of Postgate (1963):

Medium S

ascorbic acid	(Fisher)	0.12	g
sodium thioglycollate	(B.B.L.)	0.1	g
$CaCl_2$	(Fisher Certified)	0.5	g
KH_2PO_4	(Fisher Certified)	0.5	g
$FeSO_4 \cdot 7H_2O$	(Mallinckrodt Analytical)	0.5	g
NH_4Cl	(Fisher Certified)	1.0	g
Na_2SO_4	(Fisher Certified)	1.0	g
yeast extract	(Fisher)	1.0	g
$MgSO_4 \cdot 7H_2O$	(Fisher Certified)	2.0	g
sodium lactate	(Fisher)	3.5	g
Bacto-agar		20	g
Distilled Water		1000	ml
pH		7.6	

Inoculated tubes were overlaid with a 2 cm plug of the same medium and incubated at ambient temperature until there was no further increase in the number of blackened colonies (3 to 16 days).

Non-specific sulfate-reducing bacteria were enumerated by a similar deep agar tube colony count. Modifications of the medium of Ivanov and Terebkova (1959a) were employed:

Medium T

Aerobic:	Nutrient agar (Difco) plus	
	Ferrous ammonium citrate (Fisher)	0.2 g/liter
	L-cystine (Eastman)	0.2 g/liter

Medium U

Anaerobic:	Aerobic medium plus Sodium thioglycollate (Baltimore Biological Laboratory)	0.5 g/liter

Tubes of the anaerobic medium were overlaid with a 2 cm plug of the same medium. Tubes were incubated at ambient temperature until no further increase in the number of colonies was noted.

As a check on the specificity of these enumeration procedures, 10 colonies from each of the above media were inoculated as stabs into all three of the media. Tubes were inspected daily for two weeks for growth and sulfide production.

Productivity Studies Using $NaHC^{14}O_3$

Fixation of radioactive bicarbonate was used to measure productivity. The procedure was essentially that of Steemann-Nielsen (1952) as modified by Strickland and Parsons (1965). From each depth under study, 500 ml of water was added to a series of glass-stoppered 500 ml bottles. Some of the bottles were covered with black electrical tape as well as with aluminum foil (dark bottles) and some were uncovered (light bottles); usually duplicate light bottles and duplicate dark bottles were used. One or two ml ampoules of $NaHC^{14}O_3$ (supplied directly by New England Nuclear Corp. (#NES-086S) or prepared according to Strickland and Parsons (1965) using New England Nuclear Corp. #NEC-086H $NaHC^{14}O_3$), containing either 1, 5, or 10 μC were added to each bottle. Prior to the start of incubation, bottles were kept in the dark and were handled with minimal agitation. The bottles were attached to weighted lines with floats for "in situ" incubation (at the depth of collection) for a known time period (3 to 5 hr), taking advantage of the mid-day sun (1100 to 1500 hr). At the end of the incubation period, 1.7 ml neutralized formalin (Fisher Certified) was added to stop biological activity. Also, blank bottles were collected from the various depths; these were prepared by adding the formalin within one min after the C^{14} was added. The bottles were refrigerated until filtration.

After thorough shaking, aliquots (usually 25 ml) were filtered through 0.45 μ membrane filters (Millipore) with no more than 15 in of Hg vacuum, followed by a wash with 10 ml 10% synthetic seawater (Lyman and Fleming, 1940). The membranes were attached to planchets with rubber cement, dried at room temperature in a dessicator containing "Drierite" and "Ascarite", and counted using either a Nuclear

Chicago or a Baird Atomic end window gas flow detector, typically for 3 or 5 min.

The procedure outlined by Strickland and Parsons (1965) was used to determine the counting rate (R) to be expected from the entire activity of the ampoules. Aliquots of dilutions of the ampoules were mixed with a known additional amount of carbonate followed by precipitation of the carbonates with barium hydroxide. The precipitate was collected on membrane filters and counted using the same geometry as used for counting samples. Using a self-absorption correction curve, prepared with a fixed amount of C^{14} and varying "thicknesses" of $BaCO_3$, the zero thickness count was extrapolated. This count, corrected for dilutions, is equal to R.

The CO_2 fixation rate (productivity) was calculated by the formula:

$$\text{mg C/m}^3\text{/hr} = \frac{(Rs-Rb)\ (W)\ (1.05)\ (F)}{(R)\ (N)}$$

where Rs is the number of cpm of the sample; Rb is the number of cpm of the blank; W is the total inorganic carbon content of the water in mg C/m^3; the factor 1.05 allows for the fractionation of the carbon isotopes; F is a dilution factor; R is the normalized counting rate to be expected from the entire activity of the ampoule; and N is the number of hr of incubation.

On one occasion (7/29/68) 0.4 g/liter $Na_2S_2O_3 \cdot 5H_2O$ (Fisher Certified) was added to a series of light and dark bottles prior to incubation to determine if thiosulfate might be a limiting nutrient.

In order to assess the relative importance of various types of dark CO_2 fixing organisms, an experiment to test the effect of various single substrates was performed (8/16/69). The population in a water sample from 5.0 m was spun down in a refrigerated centrifuge at 5000 G for 10 min and resuspended in an equivalent amount of Basal Medium, designed to be similar to pond water, to which the various substrates were added.

Basal Medium

$MnCl_2 \cdot 4H_2O$	(Mallinckrodt Analytical)	1.0	mg
$FeCl_3 \cdot 6H_2O$	(Mallinckrodt Analytical)	1.0	mg
NH_4NO_3	(Baker Analyzed)	1.0	mg
$NaHCO_3$	(Fisher Certified)	100	mg
KH_2PO_4	(Fisher Certified)	150	mg
K_2HPO_4	(Fisher Certified)	350	mg
Synthetic seawater	(Lyman and Fleming, 1940)	100	ml
Distilled Water		900	ml

pH adjusted to 7.0 after addition of substrates.

50 ml quantities of the resuspended populations were added to 60 ml glass-stoppered bottles.

The following substrates were used: H_2 gas (Matheson) bubbled at a rate of approximately 180 bubbles/min using a capillary pipette for 30, 60, or 120 sec; $FeSO_4 \cdot 7H_2O$ (Mallinckrodt Analytical) (1 mM); NH_4Cl (Fisher Certified) (1, 5, or 10 mM); $NaNO_2$ (Fisher Certified) (1, 5, or 10 mM); sublimed sulfur (Fisher) (1, 5, or 10 mM) plus 100 ppm Tween 80 (Difco); $Na_2S_2O_3 \cdot 5H_2O$ (Fisher Certified) (1, 5 or 10 mM); glucose (Difco Certified) (1, 5, or 10 mM). The H_2 was bubbled after the addition of the C^{14} solution; all other substrates were added prior to adding the C^{14}. To one series of bottles, all of the above substrates were added (in their lowest concentrations); to another group, none of the above substrates were added. Triplicate bottles with each of the various substrates were used; 0.5 ml formalin (Fisher Certified) was added to one of each set immediately after the addition of the C^{14} to serve as a blank. One uC ampoules (pH adjusted to 7.0) were added to each bottle. After an incubation period of 3 hr in the dark in a 20 C incubator, 0.5 ml formalin was added to each bottle. Aliquots were filtered and counted as in the general productivity procedure; results are expressed in cpm per liter.

On two occasions varying numbers of a pure culture of *T. thioparus* were added to water samples prior to incubation in the presence of $NaHC^{14}O_3$ (5.0 μ C). In each case the inoculum was a four-day culture of strain #MS-5 in Medium B. On 7/28/69, to triplicate 500 ml dark bottles containing water from 4.5 and 5.0 m, 0, 10^2, 5 x 10^2, 10^3 and 5 x 10^3 cells/100 ml (determined by direct microscopic count) were added. One bottle from each set served as a blank. After "insitu" incubation for 4.0 hr, aliquots were filtered and counted as in the general productivity procedure. On 11/8/69, water from 5.5 and 6.25 m was used and 0, 10^2, 10^3, 10^4, 10^5 and 10^6 cells/100 ml were added; bottles were incubated in the dark in a 20 C incubator for 3.5 hr, after which aliquots were filtered and counted. In each case, the inorganic carbon in the water was also determined and results expressed in mg $C/m^3/hr$.

On another occasion (3/5/70) a similar experiment was performed using a pure culture of the same organism suspended in either the above Basal Medium plus 0.5 g/liter $Na_2S_2O_3 \cdot 5H_2O$ (Fisher Certified) or Medium B. To 500 ml bottles 0, 10^2, 10^4, 10^6 and 10^8 cells/100 ml were added. Bottles were incubated in the dark at 20 C for 5.0 hr. The inorganic carbon content of the media was determined and results presented in mg $C/m^3/hr$.

Oxidation of Sulfide Using S^{35}. The procedure used by Ivanov (1957) for measuring the oxidation rate of sulfide were modified slightly. In this method, water samples are incubated in the presence of S^{35}-sulfide and then the uptake of radioactive sulfur in each of various fractions (sulfide, sulfur, sulfate, cells) determined.

The radioactive solution was prepared as follows: 1.0 mC of Na_2S^{35} (New England Nuclear Corp. #NEX-29) was dissolved in 1000 ml oxygen-free (prepared by bubbling N_2 for 2.5 hr) 0.1 N NaOH (Fisher Certified). This stock solution should have an activity of 1.0 μC per ml. The stock solution was overlaid with mineral oil and stored by freezing. Just prior to use, a portion of the stock solution was diluted in oxygen-free 0.01 N NaOH, the pH adjusted to 7.5, and this working solution was sealed in 1.0 ml ampoules which were handled with minimal agitation before use.

Nominal concentrations of labelled sulfide in the working solution were either 0.1 or 0.5 μC/ml as of 6/23/69. The initial concentrations of the various labelled constituents were determined by diluting ampoules in 60 ml oxygen-free distilled water + 1.0 ml 0.1 M $CdCl_2$ (Mallinckrodt Analytical) and then subjecting this suspension to the procedure described below.

From each depth under study, 60 ml was added to a series of glass-stoppered 60 ml bottles. Light and dark bottles and bottles with 0.2 ml neutralized formalin (Fisher Certified) added were prepared; duplicates of each were used. An ampoule of the S^{35} solution was added to each bottle which was incubated "in situ" for 24 hr. The initial sulfide level (in ppm) at that depth was determined at the start of each experiment. In the bottles containing formalin, only abiotic oxidation would be expected to occur; in dark bottles without formalin, non light-dependent biological oxidation would occur as well; abiotic, non-photosynthetic biological, and photosynthetic biological oxidation would all be expected to occur in light bottles without formalin.

After incubation, 1.0 ml 0.1 M $CdCl_2$ (Mallinckrodt Analytical) was added to each bottle, precipitating any residual sulfide as CdS. The bottles were kept on ice prior to analysis which was not delayed for more than 2 hr. A flow chart illustrating the assay procedure is shown in Fig. 3. Aliquots (10.0 ml) were filtered with 0.45 μ membranes (Millipore) and washed with 5 ml distilled water.

The membranes were attached to planchets with stop-cock grease, applying only a small amount to the edges of the membranes, dried at 30 C in a vacuum desiccator over silica gel, and counted with a gas flow detector. This was count #1 and would include sulfide as CdS, elemental sulfur, and cells. The planchets were then placed in a desiccator in the proximity of a beaker of concentrated HCl for one hr. (It has been determined that this amount of time in the presence of HCl fumes was sufficient to liberate all the sulfide as gaseous H_2S.) The desiccator was connected to a 0.1 M $CdCl_2$ trap to avoid contamination of the laboratory atmosphere with radioactive H_2S. The planchets were counted again (count #2) and this would include elemental sulfur plus cells. The elemental sulfur was then extracted repeatedly with benzene; after drying, the membranes were counted again (count #3). The original filtrate was mixed with 3.0 ml 10% $BaCl_2$ (Fisher Certified) in 4.8% (v/v) HCl (Fisher Reagent). The precipitated $BaSO_4$ was then collected on 0.45 μ membranes (Millipore) and washed with distilled water. After drying, these membranes were counted as above. It was found that more complete extraction of elemental sulfur could be obtained by extracting with benzene in the filter holder prior to acid extraction; therefore, the procedure for 8/15/69 and thereafter was modified in this manner.

According to Ivanov (1968) the self absorption in CdS^{35} precipitates is significant only when the sulfide exceeds 0.5 mg/cm^2 of filter area. Because, with the size of aliquot used, the sulfide level did not exceed this amount, no self absorption correction was applied.

Results, in most cases, were expressed as cpm (-blank) and as percentage of the total cpm appearing in each fraction. Some of the results were also expressed as rates, as calculated by Ivanov (1968):

$$\text{mg S/liter/day} = \frac{r \cdot (S^{=}) \cdot 24}{R \cdot t}$$

where $(S^{=})$ is sulfide in the water sample in ppm; R = % labelled sulfide initially; r = % labelled sulfur in the product under consideration at the end of the test; and t = duration of the test in hr.

On one occasion (11/8/69), 0, 10^2, 10^4, and 10^6 cells/100 ml of *T. thioparus* were added to dark bottles containing 60 ml water from 6.2 m. The inoculum was a four-day culture of strain #MS-5 in Medium B. Duplicate bottles with formalin added and duplicate bottles without formalin were used. Bottles were incubated at 20 C for 24 hr.

A similar experiment was also performed on 3/11/70 using a pure culture of the same organism suspended in the Basal Medium used in some of the productivity experiments plus 1.0 mg/liter sulfide (Fisher Certified $Na_2S \cdot 9H_2O$). To 60 ml dark bottles with and without formalin, 0, 10^2, 10^4, 10^6, and 10^8 cells/100 ml were added. Bottles were incubated for 24 hr at 20 C. In this case, the soluble fraction was also analyzed; this was done by evaporating aliquots of the filtrate from the sulfate analysis in planchets.

RESULTS

Physical and Chemical Data

When studying the ecology of aquatic bacteria it is necessary to consider the physical and chemical characteristics of the environment. In this study, transparency, temperature, chlorinity, dissolved oxygen, pH, inorganic sulfur compounds, inorganic nitrogen compounds, phosphate, and inorganic carbon were determined.

Transparency. Water transparency, measured with a Secchi disc, is indicative of the amount of dissolved and suspended material in the water and can be used to estimate the maximum depth of effective photosynthesis. The maximum Secchi disc reading (measured between 1000 and 1400 hr) for 1968 and 1969 was 2.0 m (7/2/69) and the minimum was 0.9 m (8/24/69) (Table 1). The mean Secchi disc reading of 1.5 m corresponded to an extinction coefficient of 1.13. The data is summarized in Fig. 4; data obtained by Emery and Yentsch in 1964 and 1965 with a photometer is included for comparison (Emery, 1969). Very little light penetrated below 4 m (less than 5% of surface intensity). This lack of transparency is characteristic of eutrophic waters (Ruttner, 1966). It has been shown that green light (480-580 mμ) penetrates natural waters better than other wavelengths (Harvey, 1963; Ruttner, 1966). Thus, it is likely that the upper and lower wavelengths of visible light were even more depleted than the Secchi disc data would indicate.

Temperature. In addition to providing information about the habitat of microorganisms, temperature data can be used to estimate the degree of stratification of an aquatic environment. Temperature data for 1968 and 1969 are given in Table 2. In the mid-summer period, there was a pronounced thermal stratification. The depth of the thermocline varied between 3.5-5.0 and 5.0-6.0 m. The maximum temperature of the hypolimnion was about 20 C, while the epilimnetic waters often reached a temperature of 24-27 C. The development of the thermal stratification during the spring and summer of 1969 is shown in Fig. 5. The water column was practically isothermal until mid-June when solar heating began to warm the epilimnion more rapidly than it was mixed. The thermal stratification continued through the summer, but occasionally the epilimnion was cooled somewhat, probably by mixing with water from the upper metalimnion. This mixing correlated with periods of cool and windy weather. The prevailing wind was from the Southwest and wind speeds in excess of 10 knots were typical on the southern part of the pond. Due to the low shoreline there was very little protection from the wind. During the late summer and into the fall, a cooling trend was seen in the epilimnion (Table 2). It is interesting that on 11/7/69 the water at 6.5 m was somewhat warmer than the rest of the water column.

Chlorinity. Chlorinity and salinity data are relevant with regard to the degree of stratification of the water column and the supply of inorganic ions to the various strata of the pond. Although the chlorinities observed during 1968 and 1969 were lower than those observed by Emery (1969) during 1964 and 1965, a pronounced stratification was seen, especially during the summer (Table 3). Emery (1969) had observed a surface chlorinity ranging, in general, from 1 - 2 ppt and a chlorinity in the hypolimnion of about 3-6 ppt. If it is assumed that this is merely diluted seawater, this chlorinity would correspond to a salinity of about 1.8 - 3.6 ppt in the epilimnion and 5.4 - 11 ppt in the hypolimnion.

The decrease in chlorinity which evidently occurred during the intervening time period may be explained by mixing and subsequent flow out of the pond, coupled with a lack of any major seawater inflow during the period. It is significant to point out that there is a net discharge of water from Oyster Pond into Vineyard Sound of about 1200 - 4000 m^3/day (Emery, 1969).

During 1968, epilimnetic chlorinity ranged from 0.6 - 0.8 ppt (S‰ = 1.08 - 1.45 ppt) and that of the hypolimnion was as high as 2.47 ppt (S‰ = 4.49 ppt) (Table 3). In general, there was a sharp halocline between 4.5 and 5.5 m. During September, mixing occurred, as the epilimnion chlorinity increased slightly and a decrease in that of the hypolimnion was observed.

Apparently, complete mixing occurred during the winter of 1968-69, since the water column was essentially isohaline during March, April, and May, 1969 (Table 3).

The chlorinity during this period ranged from 0.7 - 1.0 ppt (S‰ = 1.3 to 1.8 ppt). During June, however, a distinct increase in the chlorinity of the bottom waters was observed (Fig. 6). By 6/24/69, the hypolimnion had a chlorinity of up to 1.57 ppt (S‰ - 2.86 ppt). The increasing trend continued through the summer and by 7/31/69, a maximum chlorinity of 3.94 ppt (S‰ - 7.15 ppt) was observed, whereas that of the epilimnion remained low (Fig. 6). This increase in chlorinity is likely the result of the introduction of seawater via the outlet. The halocline during the summer of 1969 was between 4.5 and 5.5 m. A very evident circulation occurred during the autumn of 1969, as by early November, the chlorinity of the entire water column was about 1.0 ppt (S‰ = 1.8 ppt) (Table 3).

Thus, a strongly developed stratification was evident during the summer period. The combined effects of a halocline and a thermocline yielded a stable water column. This stability in conjunction with the extremely high biological productivity of the water, which will be discussed below, led to a stratification of other chemical parameters, such as oxygen, pH, and sulfur compounds.

No diurnal cycle in chlorinity nor in the depth of the halocline was observed on 7/31-8/1/69, nor on 8/14/69. Such a fluctuation might be expected if a seiche were operating in the pond. However, as pointed out by Emery (1969), the periods of seiches in lakes are controlled by the basin dimensions and a seiche in Oyster Pond would have a period of about 13 min. Such a seiche would be essentially unobservable with the techniques, sampling locations, and sampling times employed.

pH. Since the CO_2 system is the primary buffer in most aquatic environments (Hutchinson, 1957; Harvey, 1963), changes in pH usually reflect alterations in the inorganic carbon content of the water. These alterations are often the result of biological activity. The pH of the water was invariably higher in the epilimnion than in the hypolimnion (Table 4). Whereas the pH in the top 3 m was extremely variable, ranging from 7.2 - 9.9, the pH of the hypolimnion was relatively constant (6.5 - 7.0 during the summer). Typically, the maximum pH occurred at a depth between 1.0 and 2.5 m; 1.5 m was very often the depth of this maximum. The pH was often minimal in the metalimnion, coincident with the thermocline and the halocline, at about 4.5 - 5.5 m, increasing slightly toward the bottom.

Dissolved Oxygen. Dissolved oxygen concentration also reflects biological activity. While green plant photosynthesis is an oxygen-yielding process, heterotrophic activity typically consumes oxygen. Dissolved oxygen data for 1968 and 1969 are shown in Table 5. High concentrations were found in the upper part of the water column (8.3 - 12.0 ppm). The water in the epilimnion was typically supersaturated with respect to oxygen (101 - 135% saturation, as computed with Mortimer's Nomogram (Hutchinson, 1957)). Oxygen supersaturation is typical of highly productive surface waters of both marine and fresh water environments (Ruttner, 1966; Harvey, 1963; Harvey, 1963). During the summer of 1968, oxygen was depleted below 4.0 m, but by late August and through September, oxygen was found deeper in the water column and on 9/22/68 a trace of oxygen was found even at 6.0 m. This correlates with salinity and temperature changes during this period.

The entire water column was well oxygenated during the spring of 1969, but by late June, when the thermal and saline stratification was well developed, oxygen was absent below 5.5 m (Table 5). By 8/15/69, oxygen was absent below 4.0 m. After this date, oxygen was observed at progressively greater depths in the pond, as the stratification weakened.

Sulfide. In the portion of the water column lacking oxygen, relatively high concentrations of sulfide were observed. The concentration of sulfide gradually increased through the hypolimnion to the bottom. The maximum concentration observed during 1968 was 50.9 ppm at 6.5 m on 8/25/68 (Table 6). During 1969, sulfide was not observed until 4/21/69; low concentrations (0.8 - 1.3 ppm) were observed at 6.5 m through early June and it was not until late June that the top of sulfide zone started to move upward in the water column (Table 6). The maximum sulfide concentration observed during the summer of 1969 was 27 - 29 ppm. It is difficult to explain the difference in sulfide concentrations between 1968 and 1969 with certainty; it may correlate with differences in productivity, temperature, precipitation, windiness, sea water flow, or a combination of factors. As mixing was initiated in late August, the top of the sulfide zone started to move downward (Table 6). Although a partial overturn had occurred in the first week of November (Emery, personal communication), it is interesting to note that sulfide was still found at a depth of 6.5 m on 11/8/69. By 11/13/69, no sulfide was detected in the water column.

Emery (1969) observed that sulfide was absent from the bottom waters during the winter of 1963-64 after a complete circulation of the water, but was present continuously from January 1964 at least until January 1966, when the pond retained its saline stratification throughout the entire year. During this two year period, there were overturns (e.g. 3/9/64), but some sulfide-bearing water was retained in the bottom waters. The pond at that time was be classified as a meromictic lake. The lack of a totally stable water column during the winter of 1963-64, and more recently (1968-69 and 1969-70), undoubtedly correlates with the somewhat lower salinity of the hypolimnion. Emery (1969) observed oxidation-reduction potentials as low as -135 mV in the hypolimnion of the southern basin.

<u>Elemental Sulfur</u>. According to Emery (1969), the top 5 - 15 cm of the sulfide zone was often milky. This

milkiness or turbidity was repeatedly observed and was shown to be due to a suspension of elemental sulfur. Up to 11.4 ppm elemental sulfur were found in a thin stratum (Table 7). Samples from deeper waters developed this milkiness on standing, exposed to air, presumably due to oxidation of sulfide to sulfur upon the introduction of oxygen.

Other Reduced Inorganic Sulfur Compounds. Thiosulfate, polythionates, and sulfite were detected in the hypolimnion but were essentially absent from the epilimnion. In general, concentrations of these intermediate sulfur species were much lower than for sulfide.

Thiosulfate was absent from the water column during the spring except very near the bottom (Table 8). During the summer of 1968 and 1969, vertical profiles were essentially parallel with those for sulfide, and maximum concentrations of 0.7 - 1.4 ppm thiosulfate-S were found at 6.0 m. The concentrations in the upper part of the sulfide zone were in the range of 0.2 - 1.1 ppm thiosulfate-S.

The method for polythionates was incapable of detecting dithionate and of distinguishing between the higher polythionates. Although other polythionates are likely to occur as well, results are expressed as tetrathionate. Polythionates were present in low, variable concentrations near the bottom of the pond (Table 9). The concentration range was from 0.0 - 1.5 ppm tetrathionate -S.

Sulfite was present in increasing concentrations from the upper hypolimnion to the bottom (Table 10). Concentrations near the upper part of the sulfide zone were very low, 0.1 - 0.2 ppm sulfite-S, and ranged up to 0.3 - 0.7 ppm sulfite-S at 6.5 m. A definite trend toward increasing concentrations of sulfite was observed during the summer of 1969.

Sulfate. Once the saline stratification was established, increasing sulfate concentrations occurred through the hypolimnion (Table 11). Epilimnetic sulfate concentrations ranged from 24 - 40 ppm sulfate-S while concentrations in the hypolimnion ranged up to 145 ppm sulfate-S.

Assuming that the bulk of the salt in the pond was derived from diluted seawater, a number of predictions can be made. If the law of constant proportions of major seawater ions is valid for seawater entering and being diluted in this environment, one would expect specific ratios between these ions. The ratio of sulfate:chloride for average seawater is 0.1394 (Sverdrup, Johnson, and Fleming, 1942). A ratio less than 0.1394 indicates a process enriching the environment with chloride or depleting the sulfate in the environment. A ratio greater than 0.1394 would be indicative of a relative enrichment of sulfate. The sulfate:chloride ratio of Vineyard Sound seawater on 6/1/70 was found to be 0.1392 - 0.1400.

On 4/21/69, the sulfate:chloride ratio was in the range of 0.13 - 0.15 with the exception of 6.0 m where the ratio was lower (0.094) (Fig. 7). A depletion of this sort in the hypolimnion was observed throughout the summer, with sulfate:chloride ratios as low as 0.076 (7/14/69 at 5.0 m) (Fig. 8). This sulfate depletion correlated with the increase of sulfide, and other reduced compounds. In intermediate waters, a sulfate enrichment of varying magnitude was observed throughout the summer. Ratios as high as 0.212 (Fig. 7) were observed; the maximum sulfate:chloride ratio on a given date was often lower than this, but invariably a peak ratio in excess of 0.16 occurred between 4.0 and 5.0 m. The depth of this sulfate enrichment corresponded to the top of the sulfide zone. The top 1 - 2 m of the pond often, but not always, were somewhat depleted in sulfate.

A diurnal periodicity in the sulfate:chloride ratio at certain depths was established. In these studies, illustrated in Fig. 10, samples taken at 2 - 3 hr intervals were analyzed for both chloride and sulfate. On 7/31-8/1/69, the ratio was relatively high and quite constant at 4.0 and 4.3 m (0.175 and 0.144 respectively). The sulfate:chloride ratio at 4.7 m showed an interesting diurnal cycle; a decreasing trend was noted after dark and there was an increasing trend during daylight hours. The maximum ratio occurred at 2100 hr (0.122) and the minimum occurred at 0900 hr (0.108). This implies that there was a net production of sulfate during the light period and a net consumption during the dark period. The fluctuation was not as strongly developed at 5.0 m. A net sulfate production during daylight hours was observed on

8/14/69 at 5.0 m but not at 4.5 m.

Inorganic Sulfur Balances. The data for various inorganic sulfur species on four days during the summer of 1969 are summarized in the form of inorganic sulfur balances in Table 12. These balances were constructed by plotting the concentrations of each constituent vs. depth and then computing the area of the curves. The area is indicative of the total amount of sulfur (mg) as that constituent. The water column was divided into 1 m^3 cubes and the total amount of sulfur observed in each m^3 was compared with the amount predicted from a sulfate:chloride ratio of 0.1394 (see Table 3). The actual sulfur observed was subtracted from the predicted amount; a positive value would indicate a depletion and negative value would indicate a sulfur enrichment.

There was usually somewhat less sulfur in the top 1 - 2 m than predicted, whereas in the intermediate waters, especially at 3 - 5 m, there was typically an enrichment. The hypolimnion, on the other hand, was depleted in total inorganic sulfur. When the results for the entire water column were compared, there was a net removal of inorganic sulfur from the water column, with the exception of 7/2/69, when there was an enrichment.

Ammonium, Nitrite and Nitrate. The distribution of soluble inorganic nitrogen species on three occasions during the summer of 1969 is shown in Fig.11 - 13.

Nitrite concentrations were low throughout the water column (2 ppb nitrite-N). With the exception of a slight suggestion of a peak at 3 - 4 m on 8/1/69, no definite statement can be made about stratification of nitrite.

Nitrate distribution was more interesting. Concentrations were low and quite variable in the top 3 m of the epilimnion (0 - 12 ppb N). A marked peak in nitrate concentration occurred at intermediate depths. The magnitude of this peak ranged from 23 - 32 ppb nitrate-N. There was a slight seasonal trend in the depth of this peak; it occurred at 4.0 m on 7/2/69, 3.5 m on 7/14/69, and 3.0 m on 8/1/69; this trend correlates with the upward trend of the top of the sulfide zone during this period. Nitrate was entirely absent in the sulfide zone.

Ammonium was present in a much higher concentration range than nitrite and nitrate. Concentrations were relatively low throughout the oxygenated zone (100-200 ppb ammonium-N). In the hypolimnion, there was considerably more ammonium (1160 - 1350 ppb NH_4^+-N) than there was in the epilimnion and the break between the two zones was well-defined. A general upward trend in the top of the ammonium-rich zone occurred during the summer of 1969. An unknown portion of the observed ammonium actually may represent amino acid nitrogen due to a limitation in the method employed. Strickland and Parsons (1964) pointed out that this lack of specificity of the method may not be a disadvantage if the data is used in discussing water fertility, since many phytoplankton utilize amino acids as their nitrogen source.

Phosphate. The distribution of soluble reactive phosphorus (phosphate) is illustrated in Fig. 12. Epilimnetic concentrations were less than 50 ppb phosphate-P, while concentrations in the hypolimnion ranged up to 600 ppb phosphate-P. The top of the phosphate-rich zone moved upward in the water column during the summer.

Inorganic Carbon. Total inorganic carbon (carbonate, bicarbonate, CO_2) was stratified during the summer; epilimnetic concentrations generally were in the range of 7.7 - 10 ppm inorganic C whereas concentrations as high as 71.3 ppm inorganic C in the hypolimnion were recorded (see Table 31-39). This stratification was not observed during the spring of 1969.

Summary of Physical and Chemical Data. The increased amounts of inorganic carbon, ammonium nitrogen, and phosphate in the hypolimnion supported the generally accepted idea that oxygen depletion in stratified waters is due primarily to decomposition of organic matter (Hutchinson, 1957; Ruttner, 1966). This also correlated with depletion of nitrate and sulfate in the hypolimnion, implying that denitrification and sulfate reduction were taking place (Redfield, 1958; Richards, 1965).

Thus, the epilimnion in the southern basin of Oyster Pond during the summer was characterized by high light intensity, relatively high temperature (22-27 C), low chlorinity (less than 1 ppt), relatively high and quite variable pH (7.4 - 9.9), oxygen supersaturation, and relatively low amounts of inorganic nitrogen species, phosphate, and inorganic carbon. Sulfate was the only sulfur species present in the epilimnion.

The hypolimnion was a drastically different environment. There was little light, lower temperature (18 - 20 C), higher chlorinity (up to 3.9 ppt), low and relatively constant pH (6.5 - 7.0), no oxygen, and high concentrations of ammonium, phosphate, and inorganic carbon. Sulfate concentrations were lower than predicted from the dilution of seawater, but high concentrations of sulfide were present (up to 30 - 50 ppm). A number of intermediate sulfur species (sulfite, polythionates, and thiosulfates) were present.

The metalimnion was characterized by a number of gradients. The thermocline and halocline were of course important. The environment had low amounts of oxygen and reduced sulfur compounds, and intermediate quantities of phosphate ammonium and inorganic carbon. At these intermediate depths, there were maximum concentrations of elemental sulfur and nitrate. The metalimnion or interface water is of particular interest since it may be inferred that a number of oxidative and reductive reactions involving inorganic sulfur compounds occur here.

<u>Thiobacilli</u>

<u>Distribution of Thiobacilli</u>. It was originally hypothesized that there would be an enrichment for thiobacilli in the metalimnion of Oyster Pond and that these

organisms would be the primary agents responsible for sulfide oxidation and chemolithotrophic productivity in this zone. *Thiobacillus thioparus*, *T. thiooxidans*, and *T. denitrificans* were enumerated by a tube dilution procedure (MPN).

Results of enumeration of thiobacilli on 7/29 and 9/8/68 are given in Table 13. On 7/29/68, no positive tubes were observed above 5.0 m. The MPN for *T. thioparus* at 5.0 m was 490/100 ml and that for *T. thiooxidans* was 80/100 ml. *T. denitrificans* was not detected. On 9/8/68, samples were collected at closely spaced intervals between 5.0 and 6.2 m. *T. thioparus* was relatively abundant throughout this portion of the water column and maximal numbers occurred at 5.6 m (2400/100 ml). Fewer *T. thiooxidans* were observed and the maximum MPN was 150/100 ml at 5.5 m. *T. denitrificans* was even less abundant and less widely distributed than *T. thiooxidans* and the maximum MPN was 90/100 ml at 5.6 m.

The MPN results for 1968 and 1969 should not be compared directly since different media were used. The results of comparisons between the media and techniques used in the two years will be presented below.

MPN results for *T. thioparus* during 1969 are shown in Table 14. On 3/27/69, when no sulfide was detected in the water column (Table 6) the highest MPN (230/100 ml) occurred at 6.5 m. On 4/23/69 and on 5/20/69, when traces of sulfide were detected near the bottom, highest numbers also occurred at 6.5 m (790 and 490/100 ml, respectively).

By 6/24/69, when considerable amounts of sulfide were detected at 6.0 and 6.5 m, highest numbers (800/100 ml) occurred at 6.0 m, the top of the sulfide zone. On 7/5/69, when sulfide was detected at 5.5 m, the MPN was 1100/100 ml at 5.5 m. On 7/31/69, when sulfide was found at 5.0 m, the MPN was 1090/100 ml at 5.0 m and was 3300/100 ml at 5.5 m. By 8/14/69, the maximal number of _T. thioparus_ was 4900/100 ml at 5.0 m; this was the highest number observed for the two sampling years. On 8/25/69, the MPN at 5.0 m was 2100/100 ml. Thus, throughout the summer, peak numbers of _T. thioparus_ occurred in a well-defined peak near the top of the sulfide zone.

MPN data for _T. thiooxidans_ during 1969 are shown in Table 15. Although the numbers were much lower than for _T. thioparus_, the same general trends were observed. In the spring, before the stratification developed, the maximum MPN (50 - 130/100 ml) occurred near the bottom. During the summer, when sulfide was present in the hypolimnion, maximal numbers occurred near the top of the sulfide zone. The maximum MPN observed was 140/100 ml.

Table 16 illustrates the MPN data for _T. denitrificans_ during 1969. _T. denitrificans_ was not observed during the summer. Maximal numbers (50 - 80/100 ml) occurred slightly deeper in the water column than _T. thioparus_ and _T. thiooxidans_.

The enumeration data above indicates that a zone of enrichment for thiobacilli occurred near the top of the hypolimnion. The predominant _Thiobacillus_ species was _T._

thioparus, with an MPN up to 4900/100 ml.

Evaluation of MPN Procedures. In an effort to obtain higher counts of thiobacilli, different media and methods were used in 1969 than were used in 1968. The media and methods were compared on a number of occasions.

The MPN procedures used for *T. thioparus* in 1968 (10 ml Medium A, static incubation) and 1969 (5 ml Medium B, shaken incubation) were compared by adding various numbers of *T. thioparus* cells to water samples (Tables 17 and 18). The MPN of the unaltered water averaged 1.36 times higher when the 1969 procedure was used. With added cells, the 1968 procedure had a mean recovery percentage of 101% and a range of 46 - 300% recovery. The 1969 procedure also had a mean recovery percentage of 101%; the range was 50 - 230%. The slightly higher MPN for the unaltered water using the 1969 procedure may have been due to the varied medium composition or to the greater provision for aeration in the 1969 procedure. However, the results were within the 95% confidence interval of the MPN. The medium used in 1969 (Medium B) had the advantage of a pH indicator which enabled quicker screening.

The MPN procedures were also compared with spread plating on Medium C (Table 18). The plating procedure yielded somewhat higher results than the MPN, especially with the unaltered water samples. Most of these colonies, however, were regarded as false positives; out of 23 colonies inoculated into Medium B and Nutrient Broth, 19 grew profusely in Nutrient Broth and none grew in Medium B. These colonies were regarded as heterotrophic bacteria capable of producing enough acid from the limited amount of organic matter present or from endogenous metabolism to change the color of the brom thymol blue from blue to yellow. Medium C was, therefore, not suitable for enumerating _T. thioparus_ in mixed cultures, but it may be a useful medium for pure culture studies.

The MPN procedures for _T. thiooxidans_ which were used in 1968 (10 ml Medium D, static incubation) and 1969 (5 ml Medium E, shaken incubation) were compared (Table 19). On the average, the MPN of unaltered water samples were 1.45 times higher with the 1969 procedure. With added cells, the recovery percentage of the 1968 procedure ranged from 11 - 130% with a mean of 48%. The 1969 procedure had a mean recovery percentage of 118% and a range of 46 - 260%.

The higher results with the 1969 procedure were fairly consistent; this may have been due to the fact that elemental sulfur was used as substrate in Medium D while thiosulfate was used in Medium E or to the greater provision for aeration in the 1969 procedure. The 1969 procedure was preferable also because the presence of the pH indicator aided in screening.

The results of a comparison between the 1968 (10 ml Medium G) and 1969 (5 ml Medium H) MPN procedure for _T. denitrificans_ are shown in Table 20. With added cells both media had a recovery percentage that averaged 43-44% and a range of 20 - 80%. Therefore, although they were equally effective, both media apparently yielded a recovery of less than 100%. The 1969 procedure was preferable because of the pH indicator.

The results of the positive controls are relevant here. As expected, T. thioparus grew well in media intended for this organism; very slight growth also occurred near the surface of tubes intended for T. denitrificans indicating the presence of a small amount of oxygen, but no gas was produced. T. thiooxidans only grew in media designed for T. thiooxidans. T. denitrificans grew almost as well in aerobic media intended for T. thioparus as in anaerobic media designed for T. denitrificans; this was expected from previous studies with this organism (Baalsrud and Baalsrud, 1954). Ideally, the T. denitrificans MPN should be subtracted from that for T. thioparus to yield a true T. thioparus MPN. However, the MPN of T. denitrificans was usually so much lower than the T. thioparus MPN that the correction was superfluous.

On numerous occasions positive tubes of the various MPN media were plated on agar media of the same composition. Pure cultures of thiobacilli were readily obtainable by this method, the taxonomy of some of these isolates will be discussed.. On occasion, viable cultures were not recovered from old tubes for T. thioparus in which the pH had fallen below 5.0. T. thioparus is rather sensitive to the acid it produces itself (Vishniac and Santer, 1957).

Negative tubes were also checked in this manner. With one exception, no thiobacilli were obtained from these tubes. One of the few tubes for _T. thioparus_ in which most of the thiosulfate was oxidized but the pH was only slightly lowered yielded a pure culture of an obligately autotrophic _Thiobacillus_ which did not drop the pH. This isolate (#MS-25) will be discussed. It is significant that a false negative was encountered in only one tube out of more than 50 which were checked. A number of cultures of heterotrophic, thiosulfate-oxidizing bacteria were isolated from tubes in which the pH had risen. These will be discussed.

No cultures of thiobacilli capable of growing in both organic and inorganic media were isolated. Their presence would have been masked by the presence of other more abundant species. No selective procedure for the facultative chemolithotrophic thiobacilli has been developed.

Thiobacillus Culture Comparison. The morphological, cultural, and major physiological characteristics of seven _Thiobacillus_ cultures are compared in Table 21. All of the cultures were small (0.5 - 0.6 μ x 1.2 - 1.8 μ), motile, gram-negative rods. With the exception of _T. thiooxidans_ #B and Oyster Pond isolate #MS-11 which were translucent, all the cultures had a creamy yellow or white color, and were raised and circular. All grew best at 20-28 C. None of the cultures grew in organic media.

Strain #MS-5 was judged to be similar to the type culture of _T. thioparus_ (#A). Neither were markedly anaerobic nor produced gas. Both grew well on thiosulfate, precipitating elemental sulfur while dropping the pH to 3.9-4.0; both showed fair growth on elemental sulfur, dropping the pH to 6.0. The pH optimum of both strains was near 7 and their range was approximately 6-8. Both conformed well to descriptions of T. thioparus (Sokolova and Karavaiko, 1968).

Strain #MS-11 and T. thiooxidans #B both grew well at pH 3-5. Neither grew as an anaerobe and neither produced gas. Both developed well on elemental sulfur and dropped the pH to 1.0-1.1 #MS-11 dropped the pH in thiosulfate media to 2.1 while #B dropped the pH to 2.6. Both of the strains conform to descriptions of T. thiooxidans (Vishniac and Santer, 1957).

T. denitrificans #C and strain #MS-17 were characterized by abundant gas production while growing anaerobically. The pH optimum of these two strains was about 7-8 and the range about 6-8. Both grew only slightly in elemental sulfur media but grew well on thiosulfate, dropping the pH below 5. Both should be classified as T. denitrificans (Baalsrud and Baalsrud, 1954).

Strain #MS-25 was similar to T. thioparus in most respects except that it did not drop the pH below 6.5. It did not grow well at an initial pH of 6.0, while T. thioparus showed fair growth at this pH. The pH produced during the oxidation of thiosulfate by T. thioparus is dependent on the products formed; when sulfuric acid is the dominant product, the pH falls, while an increased pH is seen during tetrathionate production (Sokolova and Karavaiko, 1968).

The fact that #MS-25 does not drop the pH may thus be a reflection of the particular mixture of products of thiosulfate oxidation. Although this strain does not fit into one of the well-defined species of *Thiobacillus*, it will be regarded as a strain of *T. thioparus*, since it differs in only one major characteristic.

Photosynthetic Sulfur Bacteria

Blooms of photosynthetic sulfur bacteria often develop in stratified aquatic environments (Kondrat'eva, 1965). Consequently, enrichment cultures using 1.0 ml inocula were used to establish the presence of photosynthetic sulfur bacteria in the various strata of Oyster Pond. The recovery of enrichment cultures for photosynthetic sulfur bacteria in Oyster Pond are shown in Table 22. Positive enrichment cultures for *Chlorobium* were obtained from throughout the sulfide zone. Fewer positive cultures for members of Thiorhodaceae were obtained. The distribution of the latter group appeared to be restricted to a thin layer near the top of the sulfide zone.

Within 10 days after inoculation, bottles of Medium J became green and non-motile rod-shaped to ovoid bacteria, less than 1.0 μ in diameter were observed. This description conforms to the genus *Chlorobium* (Larsen, 1953). The bottles of Medium J often developed a reddish color after a few more days, and larger (2.0 x 3.0 μ), rapidly motile cells became dominant. The morphology of these cells was similar to that of some *Chromatium* species (Skerman, 1967). Medium K became either red or green within 10 days after inoculation. The dominant organisms in this medium were morphologically identical to *Chromatium* and *Chlorobium*. Despite their overall similarity, Medium K appeared to be less selective for the green bacteria than Medium J while Medium K was slightly more selective for the red organisms. The primary selective differences between the two media may have been their pH (van Niel, 1931); Medium J had a pH of 7.3 while the pH of Medium K was 7.8.

Absorption spectra of methanol extracts ("in vitro") of the green enrichment cultures showed well-defined peaks at 435 and 665 mμ and a minor peak at 620 mμ (Fig. 15). Such a spectrum is characteristic of algae and of members of the Chlorobacteriaceae which possessed bacteriochlorophyll c (Jensen, *et al.*, 1964). Clear evidence that the dominant organisms were green bacteria was provided by the "in vivo" absorption spectra (Fig. 15). This was indicated by the lack of an "in vivo" peak in the 660-680 mμ area and the appearance of one at 750 mμ (Brock, 1966).

"In vivo" and "in vitro" (methanol) absorption spectra of a red enrichment culture are shown in Fig. 16. The "in vivo" peaks at 800 and 850 mμ and the "in vitro" peak at 770 mμ clearly place the dominant organism among the purple bacteria (Jensen, *et al.*, 1964).

When these enrichment cultures were inoculated into agar media of the same composition abundant colonies were observed after about 8 - 12 days of growth. Most colonies from green enrichment cultures were green while most of those from red cultures were red; occasional brown colonies were also observed from both media. Most of the colonies were lenticular. Since the media contained sulfide but no added organic matter except agar, both the red and the green forms were photo-lithotrophic rather than photo-organotrophic.

The obvious conclusion from the above data is that these organisms were members of the genera *Chlorobium* and *Chromatium*.

Water samples from the sulfide zone sometimes had a definite green tint. "In vivo" and "in vitro" (methanol) absorption spectra of such a sample are shown in Fig. 17. The strong absorbance of the methanol extract at 660-670 mμ, the lack of an "in vivo" peak in this region, and the strong "in vivo" peak at 755 mu, indicate that members of the Chlorobacteriaceae were primarily responsible for the coloration. The lack of "in vivo" peaks in the 800-860 mμ range and of an "in vitro" peak at 770-775 mμ, suggested that purple bacteria were not important in these water samples.

The absorbances at 660 mμ and 770 mμ of methanol extracts (10 ml extract: 100 ml sample) of water samples collected at various times were used as comparative indices of abundance of *Chlorobium* and *Chromatium* respectively (Table 23). The absorbance at 660 mμ at 1.5 m ranged from 0.188 to 0.354. The "in vivo" absorbance at 660-670 mμ was also high, indicating that this was due to algal pigments. Peak 660 mμ absorbance in the sulfide zone typically occurred at 5.5 m; this was not accompanied by high "in vivo" absorbance. The absorbance at 5.5 m ranged from 0.046 to 0.866. The 770 mμ absorbance was low throughout the water column (less than 0.07).

Using the equations of Takahashi and Ichimura (1968) and Stanier and Smith (1960) an approximation of the amount of bacteriochlorophyll c was made; the absorbance at 5.5 m would be equivalent to about 17 to 101 ppb bacteriochlorophyll c . It was assumed that the average Chlorobium cell was 10% ash and had a protein composition of about 40% of the dry weight (Redfield, 1958). According to Cohen-Bazire, et al. (1964), the chlorophyll content of Chlorobium spp. grown at low light intensities is 100-190 μg/mg cell protein, therefore a reasonable estimate of the amount of chlorophyll/cell is about 8.8×10^{-12} mg. The chlorophyll concentration observed in water samples would be equivalent to 0.19 - 1.15×10^{7} cells/ml.

Fewer than 10^{7} cells/ml were observed by direct microscopic count at 5.0, 5.5, and 6.0 m on 7/2/69. However, on 7/27/69, 5.0×10^{7} cells/ml were observed at 5.5 m. Most of these cells were small non-motile rods, conforming to the morphology of Chlorobium. Thus, many, if not most, of these cells observed microscopically were probably Chlorobium.

Enrichment Cultures for Other "Colorless Sulfur Bacteria"

It became apparent that the number of thiobacilli was insufficient to account for the observed dark CO_2 fixation and sulfide oxidation. Therefore, it was of interest to determine whether some of the other "colorless sulfur bacteria" were present in Oyster Pond. Since adequate enumeration methods have not been developed, the enrichment culture approach was utilized. Large inocula (2000 ml) were used because samples were obtained during the winter (1/15/70) after the stratification had disappeared. The letters (A - E) refer to the particular enrichment procedure employed (page 78).

Enrichment Cultures for Other "Colorless Sulfur Bacteria"

A. Both unaltered water samples (from 3.0 m and 6.5 m) which were maintained at 4 C remained oxygenated (5 - 7 ppm O_2) throughout the 8 weeks of incubation and the pH remained in the range of 7.0-7.5. The MPN of *T. thioparus* remained below 50/100 ml. The water samples examined microscopically revealed small numbers of a variety of morphological types: rod-shaped bacteria, cocci, ovoids, spirilla, and few filaments.

B. Water samples kept at 20 C became somewhat depleted in oxygen. By 8 weeks, there were 2.3 ppm O_2 in the culture from 6.5 m and 3.1 ppm O_2 in the culture from 3.0 m. The pH of both was about 7.0 - 7.2. The MPN of *T.* thioparus varied from 0-50/100 ml. Microscopically, there were slightly higher numbers of cells present than in the 4 C cultures and in addition to the above cell types, structures resembling germinating endospores were observed after about 3 - 5 days.

C. The de-oxygenating procedure (flushing with N_2)

proved to be quite effective; the oxygen content remained below 0.5 ppm O_2. Traces of sulfide were observed (less than 1.0 ppm sulfide). The pH ranged from 7.0-7.2 This technique proved to be an effective enrichment for *T. thioparus*. After 3 weeks, the MPN in the culture from 3.0 m was 230/100 ml, 4900/100 ml after 6 weeks, and 1100/100 ml after 8 weeks. In the culture from 6.5 m, the MPN of *T. thioparus* was 490/100 ml after 3 weeks, 6400/100 ml after 6 weeks, and 2200/100 ml after 8 weeks. Ovoid and rod-shaped cells were fairly abundant, as were small motile vibrios. Occasional filaments were also seen. The filaments were 1 - 2 μ in diameter and contain abundant inclusions of volutin, lipoidal material, and occasionally elemental sulfur. An inclusion was judged to consist of elemental sulfur, if it was extractable with pyridine. The filaments were gram-negative and bore a resemblance to *Beggiatoa* (Ellis, 1932; Faust and Wolfe, 1961).

D. In the *Beggiatoa* enrichment using Faust and Wolfe's technique, oxygen was depleted after 2 - 3 weeks. After 3 - 4 weeks, up to 5 - 10 ppm sulfide were observed in the enrichment cultures from both 3.0 and 6.5 m; this amount of sulfide was observed until the eighth week, by which time the sulfide level had dropped to 1 - 2 ppm. The pH was 6.8-7.0 in both cases. In the culture from 3.0 m, the MPN of *T. thioparus* was 130/100 ml after 3 weeks, 800/100 ml after 6 weeks, and 790/100 ml after 8 weeks. The MPN in the 6.5 m culture was 490/100 ml after 3 weeks, 1090/100 ml after 6 weeks, and 3300/100 ml after 8 weeks. Samples for oxygen, sulfide, and *T. thioparus* were taken from about 10 cm below the surface; since a vertical gradient probably existed, the numbers should not be considered as representative of the whole flask. As soon as the oxygen was depleted, abundant white flocculent growth appeared near the tops of both enrichment cultures; most of this material clung to the sides of the vessel and sunk to the bottom upon agitation; a white surface film developed as well.

The white flocculent material consisted of a tangled mass of filaments as well as a miscellaneous mixture of other cell types. Many of the filaments were similar to those observed in enrichment D. Most cells in the filaments were about 1.0 - 2.0 μ in diameter. Cells were typically longer than they were wide and were gram-negative. Gliding motility was sometimes observed. Movement was quite slow and could be seen most easily by noting the relative change in the positions of adjacent filaments at 5 min intervals. The cells contained both volutin and lipoidal inclusions, often appearing to be full of lipid material. Granules of elemental sulfur were seen in many of the cells; these inclusions were quite refractory and were extractable with pyridine. By their morphological characteristics, these filaments were judged to be members of the genus *Beggiatoa* (Ellis, 1932; Breed, *et al.*, 1957; Skerman, 1967).

Ensheathed filaments, about 2 μ in diameter, were observed; cells were gram-negative and no motility was observed. The sheaths were sometimes practically full of cells with only an occasional gap, and were sometimes empty or practically empty. The sheaths were occasionally impregnated with ferric iron. These filamentous bacteria were judged to be members of the Chlamydobacteriales, possibly Sphaerotilus (Skerman, 1967). The cells contained lipoidal, volutin, and sulfur inclusions. Deposition of elemental sulfur by Sphaerotilus natans had been observed by Skerman, Dementjeva, and Carey (1957).

Among the other cell types which were observed were large and small rod-shaped, ovoid, spiral, vibrioid cells. Some of the cells were rapidly motile. A variety of protozoa, including flagellates, ciliates, and amoebae were observed. When the cultures were about 8 weeks old, few filaments were observed, except those which appeared to be empty iron-encrusted sheaths, and the dominant organisms were large (2 μ x 3-8 μ), motile, gram-negative rods which contained lipoidal and volutin inclusions.

E. The enrichment technique for Thiovulum was adequate for establishing a sulfide-oxygen gradient. Sulfide concentrations near the bottom ranged from 0.5 - 3.0 ppm sulfide. Sulfide was not observed near the top of the cultures. The oxygen concentration near the surface was maintained between 2 and 4 ppm O_2; oxygen was not present near the bottom. The pH throughout the cultures from both

3.0 and 6.5 m ranged from 7.0-7.5. The MPN of T. thioparus about 2 cm from the bottom of the culture from 3.0 m was 1100/100 ml after 3 weeks and 1400/100 ml after 8 weeks; the MPN about 2 cm from the surface after 8 weeks was 230/100 ml. The MPN of T. thioparus about 2 cm from the bottom of the 6.5 m culture was 1700/100 ml after 3 weeks and 3500/100 ml after 8 weeks; the MPN about 2 cm from the surface after 8 weeks was 460/100 ml.

The cells observed microscopically in these cultures were similar in kind and number to those seen in the de-oxygenated cultures (C). Thiovulum was not observed.

The results from these enrichment cultures demonstrated that if the proper conditions were provided (presence of sulfide) T. thioparus developed, even if a mid-winter inoculum was used; the MPN of the inoculum was very low (0/100 ml at 3.0 m; 20/100 ml at 6.5 m). The conditions in enrichments C, D, and E, were similar to those in the metalimnion of a stratified pond in the spring and summer. These conditions also favored a modest enrichment of filamentous bacteria containing sulfur granules (Beggiatoa).

The presence of a small amount of added organic matter (extracted straw) appeared to heighten the natural depletion of oxygen, which occurred even in unaltered water samples which were kept at 20 C. The resultant sulfide and/or the organic matter appeared to enrich for Beggiatoa and Sphaerotilus, both of which are capable of depositing sulfur when exposed to sulfide.

The lack of development of other colorless sulfur bacteria, such as *Thiovulum*, *Thiothrix*, and *Thiodendron*, in these enrichment cultures is not definitive evidence that they are not present in Oyster Pond.

Other Chemolithotrophic Bacteria

The enrichment culture approach was utilized in order to establish the presence of other chemolithotrophic bacteria, such as methane, hydrogen, iron, and nitrifying bacteria, in Oyster Pond. While the results are not quantitative, to have a positive enrichment culture there must be at least one, and probably more, of the particular organism in the inoculum; with a 1.0 ml inoculum there must be at least one of these organisms/ml to produce a positive enrichment culture.

Methanol-oxidizing bacteria appeared to be present at all depths in the water column on both 7/5/69 and 7/27/69 (Table 24). Medium L, containing methanol, quickly became turbid and often developed a pink pellicle. Microscopic examination revealed the dominant organisms as small, motile, gram-negative rods. Organisms which utilize C_1 compounds as their sole source of energy fall into a number of groups but the most common aerobic isolates are motile, gram-negative rods with a pink pigment (Quayle, 1961). These organisms have been given many names but are commonly referred to as either *Methanomonas methanica* or *Pseudomonas methanica*. While many of these organisms can utilize the reduced C_1

compounds as sole source of carbon, some are capable of fixing large amounts of exogenous CO_2 (Quayle, 1961).

Hydrogenomonas was found primarily in the epilimnion (Table 24). Tubes of Medium M developed a slight turbidity and small, gram-negative, motile rods were observed microscopically.

Tubes of Medium N for *Gallionella* often developed a faint growth ring when inoculated with water from 3 - 5.5 m. The presence of spiral $Fe(OH)_3$ strands of *Gallionella* was not confirmed microscopically in most of these tubes. *Gallionella* appeared to be restricted to the 4.5 - 5.0 m layer of the pond (Table 24). Many of the other tubes contained rod-shaped cells embedded in a mucilaginous matrix containing ferric iron. This description conforms to the genera *Sideromonas* or *Siderocapsa*. However, the validity of these genera and their classification as iron bacteria is doubtful (Skerman, 1967).

Two tubes of Medium O for *Ferrobacillus* which were inoculated on 7/5/69 with water from 5.0 m, became rusty colored after almost 2 weeks incubation (Table 24). Virtually all the ferrous iron had been oxidized and the pH dropped to 2.5-3.0. Numerous rapidly motile, gram-negative rods were observed in both tubes. These were considered to be positive enrichments for *Ferrobacillus* (Leathen, *et al.*, 1956).

Ammonium-oxidizing bacteria were generally recovered throughout the epilimnion and the metalimnion (Table 24).

Positive tubes were indicated by the appearance of nitrite in Medium P. In some of the tubes nitrate was also detected indicating that oxidation of nitrite had occurred. Motile rod-shaped, gram-negative bacteria were observed in many of these tubes; cells of this description conform to the genus Nitrosomonas (Skerman, 1967).

The distribution of nitrite-oxidizing bacteria was more limited; positive enrichment cultures were obtained from 4.0 - 5.5 m (Table 24). A positive culture was indicated by the appearance of nitrate in Medium Q. Rod-shaped gram-negative cells were observed in these tubes and motility was not observed. These characteristics conform to the genus Nitrobacter (Skerman, 1967).

Thus, a variety of chemolithotrophic bacteria were demonstrated in Oyster Pond. It is likely that these organisms account for a portion of the dark CO_2 fixation which was observed.

Heterotrophic Bacteria

Because of their importance in the mineralization of organic matter, aerobic and anaerobic heterotrophic bacteria were enumerated, using spread plates of organic media. Some of these organisms may also be important in production of sulfide and in oxidation of inorganic sulfur compounds.

Distribution of Aerobic Heterotrophic Bacteria. The distributions of heterotrophic bacteria on 7/29/68 and 9/22/68 are shown in Table 25. On 7/29/68, when samples were taken above 5.0 m, highest numbers, in both Nutrient Agar and Medium R, occurred at 4.0 m. In Nutrient Agar, plate counts ranged from 3.0 - 7.1 x 10^3/ml and the range was 1.7 - 4.1 x 10^3/ml in Medium R. Since the number of colonies on Nutrient Agar was significantly higher than in Medium R, after 7/29/68 only Nutrient Agar was used. On 9/22/68, highest numbers occurred at 5.0 - 5.7 m, with plate counts as high as 9.8 x 10^3/ml.

The distribution of aerobic heterotrophic bacteria during the spring and summer of 1969 is shown in Table 26. On 3/27/69, the highest plate count was at 1.0 m (8.5 x 10^3/ml). By 4/23/69, a ten-fold increase in the plate count at most depths had occurred; again, peak numbers occurred at 1.0 m (8.7 x 10^4/ml). On 5/20/69, the numbers of heterotrophic bacteria were fairly uniform throughout the entire water column (1.2 - 2.3 x 10^4/ml). On 6/24/69, highest numbers occurred in the epilimnion and the maximum plate count was 1.7 x 10^4/ml at 2.0 m. On 7/5/69 and 7/15/69, maximum numbers occurred at 3.0 m (9.9 x 10^3/ml and 8.9 x 10^3/ml, respectively). On 7/31/69 and 8/14/69, highest numbers were at 4.0 m (7.7 x 10^3/ml and 8.2 x 10^3/ml, respectively). Throughout the spring and summer, highest numbers of aerobic heterotrophic bacteria occurred at progressively greater depths, until August, when the majority of the population appeared to be situated slightly above the thermocline.

During the summer stratification, the number of aerobic heterotrophic bacteria in the hypolimnion was about 50% lower than the number in the epilimnion.

<u>Distribution of Anaerobic Heterotrophic Bacteria</u>. Table 27 summarizes the data on the distribution of anaerobic heterotrophic bacteria during 1969. Plate counts were highest in the hypolimnion, where counts of 4.5×10^3/ml were often observed.

The plating procedure was compared with a deep tube colony counting procedure, which might be expected to yield more rigorously anaerobic conditions, on 7/5/69. Using this deep tube procedure, numbers were somewhat lower in the epilimnion, whereas they were approximately the same in the hypolimnion.

<u>Heterotrophic Thiosulfate Oxidizing Bacteria</u>. In a number of the MPN tubes for thiobacilli, an increase in pH to as high as 8.0 was observed. Although most of the thiosulfate in these tubes had been oxidized, the presence of thiobacilli was not demonstrable. A number of pure cultures of motile, gram-negative, rod-shaped bacteria were isolated from these tubes by subculturing into Medium I containing asparagine, peptone, glucose, or yeast extract, and by subsequently plating on agar media. Four cultures distinguishable by cultural characteristics, were selected. All were capable of oxidizing thiosulfate with an increase in pH when growing on any of the above four organic substrates.

These organisms grew well in a peptone medium without thiosulfate but did not appreciably increase the pH. When the medium was supplemented with thiosulfate, no increase in final cell number was observed and the culture oxidized the thiosulfate with a rise in pH to 8.5-9.0. No growth was observed in the absence of peptone. The results suggested that the isolates were heterotrophic bacteria with the capability of oxidizing thiosulfate to tetrathionate with no apparent utilization of the resultant energy. They were, therefore, comparable to the so-called *Thiobacillus trautweinii* (Trautwein, 1924), Starkey's cultures B, T, and K (Starkey, 1935), culture M of Parker and Prisk (1953), and to a number of species of *Pseudomonas* (Trudinger, 1967).

On 7/29/68, 100 colonies from Nutrient Agar plates from each of four depths in Oyster Pond were inoculated into a peptone-thiosulfate medium. Some of the colonies which grew in this medium oxidized thiosulfate with a rise in pH. The number of thiosulfate-oxidizing colonies from each of these depths was: at 1.5 m, 7 of 93; at 3.0 m, 2 of 98; at 4.0 m, 2 of 90; and at 5.0 m, 1 of 92. None of these 12 cultures grew on thiosulfate alone. Two were *Bacillus* sp, two were yeasts, and 8 were small, motile, gram-negative rods.

Similar results were obtained on 7/7/69, when only 25 colonies were picked from each of four depths. The numbers of thiosulfate-oxidizing colonies at these depths was: at 1.5 m, 2 of 20; at 4.5 m, 0 of 21; at 5.0 m, 0 of 20; and at 5.5 m, 0 of 16. One was a *Bacillus* and the other was a yeast. Neither grew on thiosulfate alone.

The overall percentage of heterotrophic bacteria and yeasts in the above experiments which was capable of oxidizing thiosulfate was about 3%. Heterotrophic thiosulfate-oxidizing bacteria were more abundant in the epilimnion than at 3 - 5.5 m.

Sulfide-Producing Bacteria

The dissimilatory sulfate-reducing bacteria and the non-specific sulfate reducing bacteria were enumerated using deep tube colony counts. The distribution of dissimilatory sulfate-reducing bacteria during the spring and summer of 1969 is illustrated in Table 28. On 3/27/69, although the water at 6.5 m contained oxygen (Table 5), 130 sulfate-reducing bacteria/ml were observed at that depth. By 5/20/69, the number at 6.5 m had increased to 400/ml, and 20/ml were found at 6.0 m; traces of sulfide were found at 6.5 m (Table 6). During July, 10-500/ml were found in the metalimnion and the hypolimnion. Sulfate-reducing bacteria were not found above 4.5 m in the southern basin. Highest numbers invariably occurred at 6.5 m. Higher numbers were found in the upper layers of the sediments; numbers in the sediments ranged from 1.1 x 10^4/g on 3/27/69 to 5.9 x 10^4/g on 7/15/69.

The distribution of aerobic and anaerobic non-specific sulfate-reducing bacteria on 5/20/69 and 7/15/69 is shown in Table 29. Media for these organisms (Medium T and Medium U) lacked added sulfate but contained peptone and beef extract plus additional cystine since sulfide produced by these organisms originates from peptides and amino acids.

Small numbers (7-40/ml) of aerobic bacteria of this type were found throughout the water column on 5/20/69 and 7/15/69. Somewhat higher numbers (580-620/ml) were found in the sediments. Anaerobic non-specific sulfate-reducing bacteria were not as widely distributed but were more abundant in the hypolimnion. They were not observed at 1.5 m, but up to 460/ml were found at greater depths. Numbers in the sediment were higher (4300-4700/ml).

The specificity of Medium S for the dissimilatory sulfate-reducing bacteria, Medium T for the aerobic non-specific sulfate-reducing bacteria, and Medium U for the anaerobic non-specific sulfate-reducing bacteria, was checked by inoculating colonies from each medium into all three of the media; the results are shown in Table 30. Of 10 colonies picked from Medium S, 6 grew and produced sulfide in Medium S, none produced sulfide in Medium T, and one produced sulfide in Medium U. Of 10 colonies picked from Medium T, 10 grew and produced sulfide in Medium T, two produced sulfide in Medium U, and none produced sulfide in Medium S. Of 10 colonies picked from Medium U, 9 grew in Medium U but only 8 produced sulfide, 4 produced sulfide in Medium T, and only one produced sulfide in Medium S. Apparently, some of the non-specific sulfate reducing bacteria were facultatively anaerobic. Medium S was quite specific for the dissimilatory sulfate-reducing bacteria. Medium T and Medium U were specific for the non-specific sulfate-reducing bacteria. It is likely that Medium S contained insufficient quantities of sulfur-containing organic matter for the development of non-specific sulfate-reducing bacteria, while Medium T and Medium U contained insufficient sulfate ion for the dissimilatory sulfate-reducing bacteria.

In summary, the dissimilatory sulfate-reducing bacteria were found only in the hypolimnion and the sediments, whereas the non-specific sulfate-reducing bacteria were more widely distributed. Numbers of both groups in the hypolimnion ranged up to 400-500/ml, while much higher numbers were observed in the sediments. In the sediments, numbers of dissimilatory sulfate-reducing bacteria were about one log higher than numbers of non-specific sulfate-reducing bacteria.

Dissimilatory sulfate-reducing bacteria must reduce sulfate during the oxidation of organic matter, while sulfide produced by the non-specific sulfate-reducing bacteria is merely a by-product of the degradation of sulfur-containing organic matter. Thus, more sulfide is probably produced per cell by the dissimilatory sulfate-reducing bacteria than by the non-specific sulfate-reducing bacteria. On the basis of the enumeration of the two groups of organisms, most sulfide production in the hypolimnion and in the sediments is attributed to the dissimilatory sulfate-reducing bacteria.

Productivity Studies

Although photosynthetic organisms are usually considered to be the primary CO_2 fixing organisms in aquatic environments, in areas where high numbers of chemolithotrophic bacteria are found, significant dark CO_2 fixation might occur. It was hypothesized that such chemolithotrophic productivity would occur in the metalimnion of Oyster Pond. In these experiments, $NaHC^{14}O_3$ was used to measure CO_2 fixation. Light bottle fixation of C^{14} represents the total CO_2 fixed by photosynthetic and non-photosynthetic organisms, while only non-photosynthetic fixation occurs in dark bottles; light bottle minus dark bottle fixation represents photosynthetic fixation only.

Some of the limitations of the method must be noted. It was observed by Steemann-Nielsen (1955) that the values obtained by the C^{14} procedure are intermediate between net and gross productivity. In other words, a portion of the CO_2 lost by the plankton during respiration is labelled. The method also neglects the portion of the productivity due to plankton passing through the membrane filters employed, which in this case had a pore size of 0.45 μ. In addition, a portion of the C^{14}-labelled organic materials may escape detection because it is excreted as extracellular products. The importance of carbon isotope fractionation during CO_2 fixation is not definitely established; as recommended by Strickland and Parsons (1965) it is assumed that C^{12} is incorporated at a rate 5% greater than C^{14}. Strickland and

Parsons (1965) also pointed out, as possible sources of error, the participation of intracellular carbonate in photosynthesis and the formation of carbamino complexes with CO_2 in the water under study. Nevertheless, these authors attest that most workers feel that the results approximate net CO_2 fixation and the general superiority and greater sensitivity of this method over the older method, in which evolved oxygen is measured, is widely accepted (Ruttner, 1966). In addition, the oxygen technique is valid only for typical green plant photosynthesis while the C^{14} procedure can be applied to bacterial photosynthesis and chemosynthesis as well.

For the purposes of this study, most attention was directed at the metalimnion. Consequently, samples were most closely spaced in this region. Data for 1.5 m were obtained for comparison. Maximal photosynthetic activity by epilimnetic phytoplankton was expected at approximately this depth (judging from pH data). Because only one depth in the epilimnion was sampled, it was difficult to estimate the total productivity for the entire water column.

Productivity results obtained during the summer of 1968 and the spring and summer of 1969 are presented in Table 31-39 and typical midsummer results are shown graphically in Fig. 18.

Photosynthetic productivity at 1.5 m was very high, as expected in a eutrophic lake. The range was about 20-75 mg $C/m^3/hr$, averaging 40-60 mg $C/m^3/hr$, during the summer. There was the suggestion of a seasonal cycle, with highest productivity occurring in May and June. However, too few measurements were made to be able to speculate accurately about the annual cycle and the total productivity for the year. With one exception, the highest measured photosynthetic activity occurred at 1.5 m. On 9/21/68, after a partial circulation and an extremely heavy bloom of the blue-green alga, Microcystis, maximum photosynthetic activity occurred at 4 m (108 mg $C/m^3/hr$); this was the highest productivity observed during the entire study (Table 32).

Typically, there was a decreasing amount of photosynthesis from 1.5 m to the metalimnion, but there was often a secondary maximum in the sulfide zone. This deep photosynthetic activity was observed as early in the season as 6/25/69 (Table 35), and it occurred through the summer (whenever samples in this region were spaced closely enough to reveal its presence) (Tables 31, 32, 35 and 38). The magnitude of this deep photosynthesis was as high as 40 mg $C/m^3/hr$ (6/25/69 at 6.0 m) but 10 - 30 mg $C/m^3/gr$ was more common.

The photosynthetic acitvity at depth invariably occurred in the upper part of the sulfide zone and correlated with the abundance of photosynthetic sulfur bacteria, as judged by positive enrichment cultures for Chlorobium and Chromatium (Table 22) as well as "in vivo" and "in vitro" absorption spectra of water samples (Fig. 15-17). The latter evidence especially, indicated the predominance of the green sulfur bacteria, which might be expected from the low pH and relatively high sulfide concentration (van Niel, 1931). Although the photo-organotrophs, especially *Chloropseudomonas*, cannot be eliminated from consideration, the evidence from the S^{35} studies below indicated the presence of an active flora of photosynthetic sulfur bacteria. Most of this evidence supports the conclusion that *Chlorobium* was the predominant photosynthetic organism fixing CO_2 in the sulfide zone.

Dark CO_2 fixation was very low in the summer epilimnion (Fig. 18) and this was also true for the rest of the water column during the spring (Tables 33-35). During the summer, however, high dark CO_2 fixation occurred in the metalimnion. This dark fixation usually occurred as a well-defined peak near the top of the sulfide zone (Fig. 18).

The dark CO_2 fixation maxima observed in 1968 were higher than those observed during 1969. On 7/29/68 dark CO_2 fixation increased from less than 1 mg $C/m^3/hr$ at 1.5 and 3.5 m to 26.2 mg $C/m^3/hr$ at 5.0 m (Table 31). A partial circulation occurred during September 1968, so that the epilimnion extended to almost 6.0 m on 9/21/68 (Tables 5 and 6). Epilimnetic dark CO_2 fixation was low (less than 1.2 mg $C/m^3/hr$), increasing to 63.8 mg $C/m^3/hr$ at 6.2 m (Table 32). This was the highest dark CO_2 fixation observed during the entire study. It was so much higher than the other results reported in this thesis and other dark fixation values reported in the literature that its validity is questioned. This high CO_2 fixation value may represent the activity of a sediment flora mixed with the deep water sample; samples from 6.2 to 6.5 m sometimes were mixed with flocculent sedimentary material. The proximity of the bottom may create an ecological situation different from that at higher levels of the water column, and results should not be compared directly.

During the spring and early summer of 1969, before the pond's stratification was well-established, dark fixation was low throughout the water column (Tables 33-35). On 4/22/69, dark fixation ranged from 1.2-2.1 mg C/m^3/hr (Table 33) and the range was from 0.8-2.3 mg C/m^3/hr on 5/22/69 (Table 34), and from 0.5-2.1 mg C/m^3/hr on 6/25/69 (Table 35). Obviously, sampling on 7/5/69 (Table 36) was not extended to a great enough depth and a dark maximum may have been missed; in the samples obtained, dark CO_2 fixation ranged from 0.1-1.5 mg C/m^3/hr.

By 7/19/69, a well-defined dark CO_2 fixation maximum was evident (Table 37). Dark CO_2 fixation of 15.2 mg C/m^3/hr was observed at 5.0 m, while that at 5.5 m was 19.4 mg C/m^3/hr. A similar dark CO_2 fixation peak was observed on 7/28/69 (Table 38). The magnitude at 5.0 m was 18.3 mg C/m^3/hr, while 13.6 mg C/m^3/hr was fixed at 5.5 m. On 8/24/69, peak dark CO_2 fixation of 18.9 mg C/m^3/hr was observed at 5.0 m (Table 39).

Thus, when there was a well-defined stratification in the pond, high dark CO_2 fixation occurred at the top of the sulfide zone. The magnitude of this fixation was higher than expected and it actually accounted for a major fraction of the CO_2 fixed in the water column. Assuming that photosynthetic CO_2 fixation occurs only about 12 hr per day and dark fixation occurs throughout the night as well, the importance of the dark CO_2 fixation to the carbon budget of the water column becomes more apparent.

Using the data of Tables 31, 37, 38 and 39, curves of estimated photosynthetic and non-photosynthetic CO_2 fixation for the entire water column were drawn. These estimates are only approximations. An average of about 2180 mg C/m^2/day was estimated for total photosynthetic CO_2 fixation, while the total dark CO_2 fixation was approximately 610 mg C/m^2/day, or 28% of the photosynthetic productivity.

These estimates were made for the mid-summer period, when epilimnetic photosynthesis was not as high as during spring and autumn blooms, and when dark fixation was highest. Therefore, on an annual basis, the dark CO_2 fixation is not as important as during the summer.

According to Emery (1969), the total volume of water below 4 m is only about 9% of the total volume of Oyster Pond. It would be about 26% of the total volume of a 1 m^2 water column in the deepest part of the southern basin where the measurements were made. Therefore, when considered on a whole pond basis, the reactions, such as CO_2 fixation, occurring in this deeper water, are proportionally less important. Yet, it is evident that dark CO_2 fixation is a major reaction in the carbon economy of Oyster Pond. Maximum dark CO_2 fixation did occur in the top portion of the sulfide zone at the same depth that maximum numbers of thiobacilli occurred (Tables 13-16). However, a number of other types of organisms capable of fixing CO_2 in the dark were present in this zone as well.

The results of an experiment in which thiosulfate was added to one series of light and dark bottles are shown in Table 31. The results are difficult to interpret, especially since duplicate bottles were not used. The most striking differences were at 4.5 m where the dark bottle fixation was higher with added thiosulfate (13.9 mg $C/m^3/hr$) than without (5.1 mg $C/m^3/hr$), and at 5.0 m where the light bottle fixation was higher with added thiosulfate (10.5 mg $C/m^3/hr$) than without (2.5 mg $C/m^3/hr$). Thus, thiosulfate may be a limiting nutrient to photosynthetic and non-photosynthetic organisms fixing CO_2 at these intermediate depths.

An experiment in which various substrates were added to populations from the top of the sulfide zone resuspended in a medium similar to pond water was performed. There are a number of difficulties with this sort of experiment: 1. the substrates are not mutually exclusive, e.g., the same organism might be able to grow with sulfide, thiosulfate, sulfur, and possibly even glucose; 2. the concentrations of nutrients used were merely convenient quantities and did not necessarily relate to the concentrations in the pond, and were rather high (the experiment was designed to estimate "potential activity" rather than activity "in situ"); 3. the substrates added may not be the ones which are naturally important to a group of organisms (heterotrophic bacteria are probably not growing on glucose in the pond and nitrifying bacteria might actually be growing at the expense of urea); 4. growth factors, etc., may have been left out of the "basal medium"; 5. it cannot be assumed that all natural nutrients are removed by centrifuging and resuspending in another medium; 6. endogenous metabolism is ignored; 7. the centrifuging step is certain to damage a portion of the natural flora (and fauna) and may conceivably completely eliminate one group or another; 8. the fixation in nature may depend on a particular association or sequence of organisms, which is disrupted by manipulation; 9. the dissolved gases and oxidation-reduction potential in the environment are altered. Clearly then, the results cannot be directly related to the events in the pond. Another difficulty was that the counts were rather low and the percentage error was therefore high.

In spite of the above limitations, the results in Table 40 are discussed and some conclusions drawn. The results were compared with those in which no substrates were added. In the cases of ferrous iron and nitrite, while the results did overlap slightly with those of the control, a slight inhibition of CO_2 fixation was noted. Under these conditions, the iron was not stable in solution and there was some flocculent precipitate, probably of ferric hydrates and phosphates; also the control blank was higher than most of the other blanks (960 cpm vs. 80-440 cpm). The nitrite concentrations used were higher than those observed in the pond (Fig. 11-13) and the toxic nature of the ion is well-known. Ammonium and hydrogen had no measureable effect. Sulfide and sulfur, at the 5 mM level, appeared to stimulate CO_2 fixation slightly. A definite stimulation by thiosulfate was observed; with increasing concentrations of thiosulfate, the CO_2 fixation increased. A similar but more dramatic effect was noted with methanol and glucose (Table 40).

The results suggest heterotrophic bacteria, methanol-oxidizing bacteria, and sulfur bacteria, as among the micro-organisms fixing major amounts of CO_2 in the dark at the top of the sulfide zone. Negative results, on the other hand, do not prove that other groups of microorganisms, such as nitrifying bacteria, iron bacteria, and hydrogen bacteria, are not important CO_2 fixing bacteria in this stratum.

The results in Table 41 are for an experiment conducted on 7/28/69 in which varying numbers of a pure culture of _T._ _thioparus_ were added to water from 4.5 and 5.0 m. It was shown that as many as 5 x 10^3 cells/100 ml did not measureably increase the rate of CO_2 fixation. The experiment was repeated on 11/8/69 (Table 42) with water from 5.5 and 6.2 m, with up to 10^6 cells/100 ml added. Again, increased dark CO_2 fixation was observed.

The effect of added _T._ _thioparus_ cells was tested again on 3/5/70 (Table 43). A pure culture of _T._ _thioparus_ was suspended in two different thiosulfate media rather than in pond water. Additions of 10^2, 10^4, and 10^6 cells/100 ml had no measureable effect on CO_2 fixation. When 10^8 cells/100 ml were added, on the other hand, increased CO_2 fixation occurred. In spite of the different amounts of inorganic carbon in the two media, the CO_2 fixation was quite similar (2.34 mg C/m^3/hr in the Basal Medium plus 0.4 g/l $Na_2S_2O_3 \cdot 5H_2O$; 3.02 mg C/m^3/hr in Medium B). The inoculum had been grown in Medium B.

In order to check these results, the following assumptions were made: 1. an average *Thiobacillus* cell is a cylinder 0.6 µ in diameter and 1.5 µ long; 2. this cell has a specific gravity of 1.1 and 75% of the wet weight is water; 3. the dry weight is 10% ash and the organic matter has the following composition: $(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)$ (Redfield, 1958); 4. the cell is dividing logarithmically and has a generation time of 22 hr, as determined in growth curves at 20 C in Medium B; 5. all of the *Thiobacillus* cell's carbon is derived from CO_2. Accordingly, one cell would fix 1.7 x 10^{-12} mg C/hr. If there are 10^2 cells/100 ml, then the fixation would be 1.7 x 10^{-6} mg C/m^3/hr. For 10^4 cells/100 ml, the fixation would be 1.7 x 10^{-4} mg C/m^3/hr; 1.7 x 10^{-2} for 10^6 cells/100 ml; 1.7 mg C/m^3/hr for 10^8 cells/100 ml. The agreement with the pure culture experiment with 10^8 cells/100 ml (2.34-3.02 mg/m^3/hr in Table 43) is quite good.

The maximum MPN of thiobacilli observed in 100 ml was 4900. The experiments designed to evaluate the enumeration indicated a recovery of better than 50%. Therefore, a maximum estimate would be 10^4 thiobacilli per 100 ml which could account for 10^{-4} - 10^{-3} mg C/m^3/hr.

To determine the likelihood that photosynthesis by *Chlorobium* accounted for most of the photosynthetic productivity in the sulfide zone, similar calculations were made. It was assumed that: 1. an average *Chlorobium* cell is a cylinder 0.8 μ in diameter by 1.2 μ long (Larsen, 1953); 2. the specific gravity of the cell is 1.1 and 75% of the wet weight is water; 3. the dry weight is 10% ash and the organic matter has the composition: $(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)$ (Redfield, 1958); 4. the cell is in logarithmic growth with a generation time of 24 hr (this may be a gross estimate of *Chlorobium* generation time, but a long generation time is likely, due to the poor light conditions); 5. the *Chlorobium* cell's carbon is derived entirely from CO_2. According to these assumptions, one cell would fix 2.2×10^{-12} mg C/hr. If there were 10^7 cells/ml (the maximum number observed microscopically was 5×10^7/ml (Page 123)), they could account for the fixation of 22 mg C/m^4/hr. The magnitude of the deep photosynthetic activity observed averaged from 10-30 mg C/m^3/hr (Tables 31, 32, 35 and 38). Thus, *Chlorobium* could account for the majority of the sulfide zone photosynthetic activity.

Sulfide Oxidation Studies

Since a pronounced stratification of sulfur compounds was observed in Oyster Pond, as well as a varied flora of microorganisms capable of oxidizing inorganic sulfur compounds, it was desirable to estimate the relative importance of the various groups of organisms vs. non-biological mechanisms in the oxidation of sulfide. Oxidation studies using S^{35}-labelled sulfide were performed. This method is capable of distinguishing between abiotic, photosynthetic, and non-photosynthetic biological oxidation. Oxidation in the presence of formalin represents abiotic oxidation only; dark bottle oxidation in the absence of formalin includes non light-dependent biological oxidation as well; light bottle oxidation in the absence of formalin includes abiotic, non-photosynthetic, and photosynthetic biological oxidation.

The results are shown in Tables 44 and 45 and the data are illustrated in monograph form in Fig. 19 and 20. In each case, the time period was 24 hr. The results are expressed in percentage of the total cpm occurring in each fraction: sulfide, "cells", elemental sulfur, sulfate, and a soluble fraction. The ampoules contained 90.4-95.7% sulfide, with minor amounts of "cells", sulfur, and sulfate. In most cases the soluble fraction was determined by difference. This fraction was likely to have included sulfite, polythionates, and thiosulfate, since these ions were not precipitated by treatment with $CdCl_2$ or $BaCl_2$.

Before considering the results, the problems with this method are presented: 1. the presence of impurities in the original sulfide is almost unavoidable (Ivanov, 1957); 2. this is not a static situation and it is possible that a portion of the originally oxidized sulfide may be reduced again; it will be assumed that the bulk of the labelled sulfur remained in the oxidized state; 3. oxidation of sulfide may proceed in a stepwise manner; this would be difficult to assess; 4. it is not known what portion of each of these fractions is intracellular; it is possible that a large percentage of the sulfur is inside the cells; 5. in cases where the cpm are low, the percentage of error is higher.

On 7/5/69, the cellular sulfur fraction was high (4.1 - 17.1%) probably, as subsequent experiments indicated, because the extraction procedure for elemental sulfur was at fault (Table 44). Much of this cellular sulfur fraction was likely to be elemental sulfur. On 8/15/69 and later, an improved extraction procedure was used and the cellular sulfur fraction was considerably smaller (0.4 - 2.3%) (Tables 45-47). Only a small portion of the sulfide became incorporated into cells.

At 1.5 m on 7/5/69, sulfide oxidation was almost complete; only 1.4 - 2.9% of the label appeared as sulfide after 24 hr (Table 44). The bulk of the product was sulfate (73.4 - 77.0% of the S^{35}). The differences between results in light and dark bottles and those with and without formalin were minor. The sulfate fraction was slightly smaller (by 1.5 - 3.6%) in the presence of formalin than in its absence, and the soluble fraction was slightly larger with formalin than without. Thus, epilimnetic sulfide oxidation was almost entirely non-biological. This conclusion was expected from the high oxygen concentration and lack of reduced sulfur compounds at this level. The lack of sulfide precludes calculations of the actual rate of sulfide oxidation.

At 5.0 m, the differences were more significant (Table 44). Light and dark abiotic oxidation differed somewhat. In the dark 19.8% remained as sulfide after 24 hr compared to 17.6% in the light. The "cells" plus sulfur fractions totalled somewhat more in the dark than in the light (39.0% compared to 32.1%). Also, the sulfate fraction in the dark was smaller than that in the light (35.3% compared to 43.1%). The soluble fraction was about 6% in both cases. Thus, there may have been a stimulatory effect of light on abiotic oxidation of sulfide and sulfur. This apparent stimulation was not seen at 6.0 m (Table 44) nor on 8/15/69 at 5.0 m (Table 45).

The oxidation rates at 5.0 m were higher in the absence of formalin than abiotically. In the dark, 12.3% of the S^{35} appeared as sulfide, 33.3% in the "cells" plus sulfur fractions, 4.4% soluble, and 50.1% as sulfate (Table 44). Further oxidation of sulfide and soluble sulfur compounds, mostly to sulfate had occurred. In the light, 7.9% appeared as sulfide, 24.5% in the "cells" plus sulfur fractions, 2.6% in the soluble fraction and 65.0% as sulfate, indicating increased oxidation of sulfide, sulfur, and soluble sulfur compounds, largely to sulfate (Table 44).

When oxidation rates at 5.0 m were calculated from data in Tables 6 and 44, dark abiotic oxidation of sulfide in 24 hr amounted to 0.436 mg S/liter; about one-half of the product was sulfate, about one-half was probably sulfur, with a small amount of soluble products. Photosynthetically, 0.004 mg sulfide-S/liter were oxidized, but sulfate production was greater (0.039 mg S/liter). Of this sulfate, 0.008 mg S/liter resulted from oxidation of soluble sulfur compounds and most of the rest probably from sulfur oxidation. Non-photosynthetic biological sulfide oxidation amounted to 0.050 mg S/liter, but, again, sulfate production was greater (0.080 mg S/liter). Apparently, about 10% of the sulfate resulted from oxidation of soluble compounds and the rest probably from sulfur.

At 6.0 m, the differences between the rates and products of abiotic oxidation in the light vs. in the dark were minor (Table 44). In either case, the sulfide fraction amounted to about 10%, the "cells" plus sulfur fractions totalled 5.7 - 5.8%, the soluble fraction was 68%, and the sulfate fraction was about 10%. Thus, although a relatively large proportion of the sulfide was partially oxidized, a major fraction of it was not oxidized to sulfate. A large sulfite, thiosulfate, plus polythionates fraction appeared instead.

Dark oxidation in the absence of formalin at 6.0 m yielded the following totals: sulfide 8.1%, "cells" plus sulfur 7.5%, soluble sulfur compounds 67.5%, and sulfate 16.9% (Table 44). There was biological oxidation of sulfide leading to slightly greater amounts of sulfur and sulfate.

These totals compare to the following totals in the light in the absence of formalin: sulfide 6.5%, "cells" plus sulfur 6.1%, soluble sulfur compounds 68.1%, and sulfate 19.3% (Table 44). These results indicate that some photosynthetic oxidation of sulfide and sulfur, largely to sulfate, took place.

At 6.0 m much more sulfide was present than at 5.0 m (Table 6), Consequently, the rate of sulfide oxidation was proportionally higher. Yet a smaller portion of this sulfide was completely oxidized to sulfate, possibly due to less communication with oxygenated surface water. Using data from Tables 6 and 44, dark abiotic oxidation at 6.0 m yielded 8.78 mg sulfide oxidized/liter in 24 hr, 1.59 mg S/liter to sulfate and the majority (6.62 mg S/liter) to soluble sulfur compounds. Somewhat higher oxidation rates were observed in the absence of formalin than in the presence of formalin. Photosynthetic oxidation of sulfide amounted to 0.23 mg S/liter, but 0.30 mg sulfate-S/liter and 0.05 mg soluble S/liter were produced. Most of this difference was probably due to oxidation of elemental sulfur. Non-photosynthetic biological oxidation of sulfide amounted to 0.15 mg S/liter, but only one-third of this resulted in the production of sulfate; most of the rest of this sulfide probably was oxidized to elemental sulfur. This is indirect evidence for the activity of organisms oxidizing sulfide only as far as sulfur. Also, 0.06 mg S/liter of soluble sulfur compounds were oxidized.

On 8/15/69 at 5.0 m, the difference between abiotic oxidation in the light vs. in the dark were relatively small (Table 45). After 24 hr, the following totals were observed: sulfide about 20.4%, cellular sulfur about 1.5%, sulfur 11.0 - 12.2%, soluble sulfur compounds about 21% and sulfate about 45%. In the absence of formalin, the oxidation rates were somewhat higher than in its presence. In the dark, the sulfide fraction constituted 16.1%, cells 2.3%, sulfur 8.8%, soluble sulfur compounds 19.6%, and sulfate 53.2%. Apparently, further oxidation of sulfide, sulfur, and soluble sulfur compounds resulted in increased amounts of sulfate. In the light, the totals were: sulfide 13.6%, cells 2.1%, sulfur 5.0%, soluble sulfur compounds 14.9%, and sulfate 63.5%. This increased oxidation of sulfide, sulfur, and soluble sulfur compounds, largely to sulfate, was attributed to photosynthetic activity.

From data in Tables 6 and 45, calculations indicated that in 24 hr, dark non-biological sulfide oxidation amounted to 4.00 mg S/liter; of this 2.24 mg S were oxidized to sulfate, 0.61 mg S to elemental sulfur and 1.07 mg S to soluble compounds. Photosynthetic sulfide oxidation amounted to 0.17 mg S/liter, while sulfate production in the amount of 0.37 mg S/liter was observed. Most of this additional sulfate apparently resulted from oxidation of soluble sulfur compounds. Non-photosynthetic biological activity resulted in the oxidation of 0.18 mg sulfide-S/liter, while 0.41 mg sulfate-S/liter were formed. The additional sulfate resulted from the oxidation of both sulfur and soluble compounds.

In summary of this data (Fig. 18 and 19), photosynthetic oxidation of sulfide in 24 hr at 5 - 6 m during the summer ranged from 0.895 - 4.03% of the amount oxidized abiotically; sulfate production by photosynthetic means was quantitatively more prevalent (2.03 - 19.6% of the abiotic rate). Non-photosynthetic biological oxidation of sulfide ranged from 1.15 - 4.50% of the abiotic rate, while sulfate production ranged from 3.14 - 18.3% of the abiotic rate. Because the effective light period at these depths was probably less than 12 hr, and since non-photosynthetic oxidation occurred in the dark as well, on an hourly basis during daylight hours rather than on a daily basis, photosynthetic oxidation was considerably more important than non-photosynthetic oxidation.

The data from these experiments showed that with (Table 6; Fig. 19 and 20) increasing sulfide concentration in the water, the soluble fraction increased and proportionally less sulfate resulted by abiotic means. Photosynthetic and non-photosynthetic oxidation rates were quantitatively equivalent and neither contributed a large proportion to the total oxidation rate. In both photosynthetic and non-photosynthetic oxidation, sulfide, sulfur, and soluble sulfur compounds were oxidized.

When varying numbers of a pure culture of *T. thioparus* were added to water from 6.2 m on 11/8/69 (Table 46 and Fig. 21) and the bottles were incubated in the dark, very little difference was observed between bottles with different numbers of added cells and between bottles with and without formalin. Thus, as many as 10^6 cells/100 ml did not measureably increase the oxidation rate. After 24 hr, 36.3 - 38.9% remained as sulfide, 10.4 - 13.9% appeared as sulfur, 6.6 - 9.6% as soluble sulfur compounds, and 38.0 - 42.4% as sulfate.

On 3/11/70, this type of experiment was repeated, except that the thiobacilli were added to a synthetic medium containing 1.0 ppm sulfide rather than pond water (Table 47 and Fig. 22). Again, in the presence of up to 10^6 cells/100 ml, no measureable increase in the oxidation of sulfide was observed. Considerably greater oxidation rates were observed when 10^8 cells/100 ml were added. In the presence of 10^8 cells/100 ml, distinct differences were noted between bottles with and without formalin. In 24 hr, in the presence of formalin, the following totals were observed: sulfide 17.6%, sulfur 23.3%, soluble sulfur compounds 10.6%, and sulfate 47.7% (Table 47). In the absence of formalin, the totals were: sulfide 15.2%, sulfur 20.1%, soluble sulfur compounds 1.8%, and sulfate 61.4%. Clearly, biological oxidation of sulfide, sulfur, and especially soluble compounds, largely to sulfate, had occurred.

When oxidation rates were calculated from calculations of data in Tables 6 and 47, it was found that in the 10^8 cells/100 ml control, abiotic oxidation of sulfide in 24 hr amounted to 0.911 mg S/liter; of this, 0.528 mg S was oxidized to sulfate, 0.258 mg to sulfur, and 0.107 mg to soluble compounds. In the absence of formalin, an additional 0.151 mg sulfate-S/liter were formed, 0.027 mg from sulfide, 0.036 from sulfur, and 0.087 from soluble sulfur compounds.

The results were reconsidered by calculating the amount of sulfide oxidation that *Chlorobium* and *Thiobacillus* spp. account for. The calculations were similar to those in the productivity section (pages 147- 148). It was estimated that the population of *Chlorobium* might account for a productivity of 22 mg $C/m^3/hr$ (page 148). Larsen (1952) showed experimentally that for every mg of carbon fixed by *Chlorobium limicola*, 1.69 mg sulfide were oxidized, while the ratio was 1:1.52 for *C. thiosulfatophilum*. Although his conditions were different from those in Oyster Pond, a mean S:C ratio of 1:1.6 was assumed. Then, the population of *Chlorobium* would oxidize about 0.84 mg sulfide-S/liter in one day. Experimental results for 5 - 6 m during the summer ranged up to 0.23 mg sulfide S oxidized/l/day and up to 0.37 mg sulfate S formed/l/day. Thus, much of the sulfide oxidized by light-dependent biological activity may have been accomplished by photosynthetic sulfur bacteria.

When similar calculations were made for thiobacilli, their significance was less evident. It was estimated that the *Thiobacillus* population would account for a productivity of 1.7×10^{-4} mg $C/m^3/hr$ (page 147). Assuming an efficiency of about 6 - 9% (Baas-Becking and Parks, 1927), and a requirement of 118 k cal for every mole of CO_2 reduced to (CH_2O), this is equivalent to a caloric requirement of about 5 x 10^{-2} cal/m^3/hr. The complete oxidation of 1 mole of HS^- to H_2SO_4 yields 160 k cal (Sokolova and Karavaiko, 1968), therefore, about 3.13 x 10^{-4} mM sulfide S/m^3/hr would have to be oxidized. This is equivalent to about 2.4 x 10^{-4} mg S/liter/day, obviously much less than the observed dark biological sulfide oxidation at 5 - 6 m during the summer (0.05 - 0.18 mg sulfide S/liter/day). By these same assumptions, 10^8 thiobacilli/100 ml would oxidize 2.4 mg sulfide S/liter/day. When this number of thiobacilli were present (3/11/70; Table 47), a sulfide oxidation rate of 0.151 mg/liter/day was observed, or about one log lower than the estimated rate. Such a discrepancy could be explained by efficiency greater than 6 - 9%, counting error, or essentially adverse conditions for the added thiobacilli. It should be noted that the inoculum was grown on thiosulfate in Medium B, while the experiment was conducted in a sulfide medium. Also, Sokolova and Karavaiko (1968) and Hempfling and Vishniac (1967) have demonstrated energy transfer efficiencies greater than 6 - 9%.

The results of the sulfide oxidation studies indicated that the population of thiobacilli observed in Oyster Pond was insufficient to account for the observed rates of non light-dependent biological oxidation of sulfide. The activities of other non light-dependent organisms, such as other colorless sulfur bacteria and heterotrophic bacteria are suggested. On the other hand, the population of _Chlorobium_ was sufficient to account for the observed rates of light-dependent biological sulfide oxidation.

Sediment Analyses

The sediments at station were analyzed in order to gain some insight into the interactions between the sediments and the overlying water column with regard to sulfur biogeochemistry.

The sediments at station 18 were fine grey to black organic muds, with an admixture of sand. Occasionally, pieces of cinders, twigs, etc., were found. The sediments had a distinct sulfide smell and were 1-3 C cooler than the overlying pond water during the summer.

The results of the analyses of sediments collected on 8/15/69, 11/13/69, and 1/15/70 are shown in Table 48. The sediments were 74.8 - 77.0% water. Organic matter accounted for 13.3 - 17.2% of the dry weight; a general seasonal increase in organic matter was observed. The chlorinity was somewhat lower on 1/15/70 (12.0 ppt) than on 8/15/69 or 11/13/69 (14.5 - 14.9 ppt).

On 8/15/69, 453 ppm sulfate-S were observed. Therefore, the sulfate:chloride ratio was 0.090. There were 246 ppm free sulfide and 439 ppm S acid-soluble sulfide. Pyrite-S amounted to 1680 ppm and there were 10.2 ppm elemental sulfur. The ratio of total inorganic sulfur (as $SO_4^=$):chloride was 0.569. When compared with the $SO_4^=$:Cl^- ratio of seawater (0.1394) there was an apparent excess of 2135 ppm sulfur in the sediment (Table 48).

The sediments collected on 11/13/69 were only partially analyzed. The observed 527 ppm sulfate-S yielded a sulfate:chloride ratio of 0.109. There were 110 ppm free sulfide and 183 ppm acid-soluble sulfide, significantly less of these sulfur forms than on 8/15/69 (Table 48).

On 1/15/70, there were 432 ppm sulfate-S in the sediment, yielding a sulfate:chloride ratio of 0.108, quite similar to that on 11/13/69. The free sulfide and acid-soluble sulfide content was also similar to that observed on 11/13/69 (109 ppm and 209 ppm, respectively). There were 1760 ppm pyrite-S and 8.7 ppm elemental sulfur. The ratio of total inorganic sulfur (as $SO_4^{=}$):chloride was 0.629, yielding an excess of 1962 ppm sulfur (Table 48).

There are at least two alternative explanations for this excess sulfur in the sediments: 1. not all of the sulfide minerals are derived from the reduction of sulfate, but some may be contributed from the dissimilation of sulfur-containing organic matter; 2. there is a net deposition of metallic sulfides from the water column.

Results from the North Basin

In order to provide supporting data for the studies in the southern basin, a similar, but abbreviated, study was conducted at station 3, the deepest part of the northern basin (4.2 m).

The transparency of the water, as seen in Table 49, was similar to that in the southern basin. The mean Secchi disc reading was 1.4 m. A pronounced thermal stratification was observed at this station (Table 50). The maximum epilimnetic temperature observed was 27.0 C, and the bottom temperature was never higher than 19.4 C. The thermocline depth was typically at 3.5 - 4.0 m. The chlorinity was low 0.6 - 0.9 ppt) and no vertical stratification was observed (Table 51). Although this shallower basin did not have a halocline like that in the southern basin, a well-developed summer thermocline resulted in a stratified water column. This part of the pond is more protected from the wind due to the surrounding topography and this heightened the stability of the water column.

The results of pH determinations appear in Table 52. The epilimnetic pH, like that in the southern basin, was quite variable (7.7 - 9.6) but the pH of the hypolimnion was lower and more constant (6.4 - 6.9). The pH maximum was observed at 0.5 - 1.5 m. A pronounced oxygen stratification was observed as well (Table 53). Dissolved oxygen concentrations in the epilimnion ranged up to 10.38 ppm (7/27/69 at 1.0 m). By late July, oxygen was depleted below 3.5 m. Inorganic carbon was also stratified (see Tables 62 and 63) increasing from about 8 ppm inorganic C at 1.5 m to as much as 25 ppm inorganic C at 4.0 m on 7/28/69. Sulfide, up to a maximum of 17.2 ppm (8/24/69 at 4.2 m), was present in the hypolimnion during the summer (Table 54). This sulfide concentration was somewhat lower than the maximum observed in the southern basin.

Other reduced sulfur compounds were observed as well. A milky turbidity was sometimes seen at about 4.0 m; on 7/27/69, 3.4 ppm elemental sulfur were detected at this depth. On 7/27/69 and on 8/24/69, thiosulfate and polythionates were detected in the water column (Tables 55 and 56). Up to 0.6 ppm thiosulfate-S and up to 0.5 ppm tetrathionate-S were observed. Sulfite concentrations are shown in Table 57; a maximum of 0.15 ppm sulfite-S (7/27/69 at 4.2 m) was detected.

The distribution of *T. thioparus* is shown in Table 58. While *T. thioparus* was not detected above 4.2 m on 7/5/69, by 7/31/60 a few (20/100 ml) were found even at 1.5 m. The maximum MPN in the water column ranged from 130 - 220/100 ml. A maximum MPN of *T. thiooxidans* of 50/100 ml was observed (Table 59). *T. denitrificans* was detected only on 8/25/69, with a MPN of 20/100 ml at 4.2 m (Table 60). The number of thiobacilli in the northern basin was considerably lower than in the southern basin, but their distribution was generally similar.

Although enrichments for photosynthetic sulfur bacteria were not made, their presence was likely. The water at 4.2 m was quite green on 7/28/69 and 1.0 x 10^7 cells/ml were observed by direct microscopic count (compared to 5.0 x 10^7 cells/ml at 5.5 m in the southern basin). Most of these cells were small, non-motile rods and thus corresponded to the morphology of *Chlorobium*. The presence of members of Chlorobacteriaceae at 4.2 m on 7/27/69 was confirmed by the strong absorbance of a methanol extract at 660 mμ (the absorbance/100 ml water using a 10 ml extract was 0.394). There was no "in vivo" peak in this region of the spectrum. The "in vitro" absorbance at 770 mμ was also low, suggesting that purple bacteria were not abundant.

The distribution of heterotrophic bacteria is shown in Table 61. While maximum numbers of aerobic heterotrophic bacteria occurred in the epilimnion (maximum plate count of bacteria 9200/ml), highest plate counts for anaerobic heterotrophic bacteria occurred deeper in the water column (2100/ml at 3.0 m on 7/5/69).

Data for sulfate-reducing bacteria are shown in Table 62. Numbers of sulfate-reducing bacteria in the water column were highest at 4.2 m (tube colony count = 400/ml on 7/5/69) and were higher in the sediment (9800/g on 7/5/69). The numbers were slightly lower than in the water and sediments of the southern basin.

Productivity data for 7/5/69 are shown in Table 63. The dark fixation was low (less than 0.6 mg $C/m^3/hr$) at all depths studied, while the photosynthetic productivity was relatively high (33.6 mg $C/m^3/hr$) at 1.5 m, decreasing toward the bottom. On 7/28/69 (Table 64) the photosynthetic productivity was again highest at 1.5 m (49.2 mg $C/m^3/hr$) and decreased toward the bottom. The photosynthetic activity at 4.0 m may represent the activity of photosynthetic sulfur bacteria since sulfide was found at this depth (Table 54). Dark fixation increased from 0 mg $C/m^3/hr$ at 1.5 m to 20.0 mg $C/m^3/hr$ at 4.0 m.

Although the northern basin is shallower and does not exhibit a saline stratification, it is similar to the southern basin in other respects. There was a similar stratification of temperature, pH, dissolved oxygen, carbonates, and reduced sulfur compounds. The lower numbers of thiobacilli may have been a result of the somewhat lower concentrations of sulfide and other reduced sulfur compounds. This, in turn, may have reflected the lower salinity (and consequently lower sulfate content) of the water. The high dark CO_2 fixation at 4.0 m on 7/28/69 (Table 63), in spite of the low numbers of thiobacilli (Tables 58-60), suggests the importance of other groups of non-photosynthetic CO_2 fixing microorganisms.

DISCUSSION

During the summer, Oyster Pond displays all the characteristics of an anoxic basin. The combined effects of the halocline, the thermocline, and the high biological productivity lead to a thoroughly stratified aquatic environment. The epilimnion is characterized by oxygen supersaturation, high and variable pH, and low concentrations of inorganic nutrients. The hypolimnion lacks dissolved oxygen, has a lower and relatively constant pH, and contains high concentrations of inorganic nutrients. While the only inorganic sulfur species present in the epilimnion is sulfate, the hypolimnion contains high concentrations of sulfide and measurable concentrations of thiosulfate, polythionates, and sulfite. The metalimnion is characterized by a number of gradients: temperature, salinity, oxygen, pH, carbonates, phosphate, ammonium, and reduced sulfur compounds, and by the presence of colloidal elemental sulfur. The metalimnion is thus a primary zone of oxidative and reductive processes of the various nutrient cycles.

Since most of the sulfate is derived from diluted seawater, the sulfate:chloride ratio provides a comparative index (Fig. 7 and 8). The sulfate depletion in the hypolimnion is explicable by the occurrence of sulfate reduction in this zone. Such a depletion was observed by Yoshimura (1932) in the hypolimnia of Lakes Hiruga and Suigetu, by Koyama and Sugawara (1953) in the hypolimnion of Lake Aburagafuchi, by Emery and Rittenberg (1952) in sediment cores from the California basins, and by Emery (1969) in Oyster Pond. The hypolimnetic sulfate depletion in Oyster Pond correlated with the occurrence of sulfide and other reduced sulfur species. But the total amount of inorganic sulfur in the hypolimnion was considerably lower than predicted, except on 7/2/69, when the total inorganic sulfur equalled the amount expected from seawater sulfate (Table 12). It is likely that some of the sulfide was removed from the hypolimnion by upward diffusion or by precipitation.

The metalimnion was somewhat enriched in sulfate, though the magnitude of the enrichment varied considerably (Fig. 7 and 8). Sulfate enrichments were observed by Yoshimura (1932) in the metalimnion of Lake Suigetu, but not Lake Hiruga, by Koyama and Sugawara (1953) throughout the metalimnion and epilimnion of Lake Aburagafuchi, and by van Gemerden (1967) in the metalimnion of Lake Pluss in northern Germany. Since the metalimnion of Oyster Pond was enriched in total inorganic sulfur compounds (Table 12), it is likely that a portion of the sulfide formed by sulfate-reduction in the hypolimnion diffused upward and was oxidized in the metalimnion.

The low sulfate:chloride ratio often observed in the top 1-2 m of the epilimnion (Fig. 7 and 8) is difficult to explain. A similar phenomenon was observed by Yoshimura (1932) in the epilimnion of Lake Suigetu. In aerobic seawater, sulfate is usually regarded as a conservative nutrient (Harvey, 1963). Emery (1969) reported a sulfate:chloride ratio of local groundwater of 0.445 and a sulfate content of only 16 ppm. According to Junge (1960) the mean sulfate content of rainwater over land is 2.2 ppm while the sulfate content of rainwater over the oceans averages 0.5 ppm. Junge reported that the sulfate:chloride ratio of rainwater is considerably greater than that of seawater, especially over land areas. Therefore, groundwater and rainwater would increase the sulfate:chloride ratio rather than decrease it.

A possible explanation for the sulfate depletion in the epilimnion is photosynthetic demand. All cells contain organic sulfur, mostly in the form of amino acids. Kaplan, *et al*. (1963) found the total sulfur content of marine organisms (excluding shells) to average 1.15% dry weight for algae and 0.89% dry weight for zooplankton. Vinogradov (1953) reported that marine crustacea and fish average 0.82% and 0.78% sulfur, respectively. Schuette (1918) observed that the sulfur content of Lake Mendota plankton averaged 0.59%, while Schuette and Adler (1927) observed that the sulfur content of rooted plants of Lake Mendota was 0.85% for *Valisneria* and 0.82% for *Potamogeton*. Roberts, *et al*. (1955) reported that *Escherichia coli* cells are about 1.12% sulfur. Using the above data, average organic matter was estimated to be 0.88% sulfur. Richards (1965) had proposed that average organic matter has the empirical formula, $(CH_2O)_{106}(NH_3)_{16}\ H_3PO_4$. A formulation including sulfur is proposed here: $(CH_2O)_{106}$ $(NH_3)_{16}(H_3PO_4)(H_2S)$. By this formula the ratio of carbon: sulfur (by weight) is 39.8.

The mean primary productivity at 1.5 m on 7/5/69, 7/19/69, 7/28/69, and 8/24/69 (Tables 36-39) was 49.2 mg $C/m^3/hr$. If it is assumed that this represents the average productivity in the top 2 m of the water column during the summer, and that there are 12 hr of effective photosynthesis per day, then the productivity in the top 2 m^3 of the water column is 1180 mg C/day. The photosynthetic requirement for sulfate-S in the top 2 m^3 would be 29.5 mg S/day.

Considering the stability of the water column, it was assumed that the top 2 m is readily exchanged while the remainder of the water column is a nutrient trap. The volume of water above 2 m is about 63% of the total volume of Oyster Pond or 470,000 m^3 (Emery, 1969). Emery estimated the average daily discharge from the pond (outflow plus evaporation) to be 3000 m^3. Therefore, the residence time of water in the top 2 m was estimated to be 157 days. During this period of time, there would be a photosynthetic requirement of about 4670 mg sulfate-S. The depletion of sulfate-S observed in the top 2 m ranged from 4200-7100 mg S, except on 8/15/69, when an excess of 2400 mg S was observed (Table 12). This change in epilimnetic sulfate content between 8/1/69 and 8/15/69, may have been due to mixing with sulfate-rich water from below. Such partial mixing occurred occasionally during the summer, as judged by temperature and chlorinity data (Tables 2 and 3).

The maximum sulfide concentration in the hypolimnion of Oyster Pond ranged from 12.5-50.9 ppm during the summer

(Table 6). This was a higher concentration than that observed in such marine anoxic basins as the Cariaco Trench (Richards and Vaccaro, 1956), Lake Nitinat, British Columbia (Richards, et al., 1965), and the Black Sea (Zenkevich, 1963), but it was lower than that observed by Takahashi and Ichimura (1968) in some Japanese brackish lakes. Among the factors which affect the actual sulfide concentration of anoxic environments are stability of the water column, availability of sulfate, and availability of organic matter. Although not permanently stratified, Oyster Pond has a fairly stable water column and an abundance of both sulfate and organic matter.

Most other investigators who have worked on similar stratified aquatic environments have not reported concentrations of intermediate inorganic sulfur compounds, such as sulfite, polythionates, thiosulfate, and elemental sulfur (Hutchinson, 1957). Intermediate sulfur compounds averaged 1.45% of the total inorganic sulfur content of the water column of Oyster Pond during July and August, 1969 (Table 12). Sulfide averaged 5.76% of the total during the same period (Table 12). The contribution of reduced and intermediate sulfur compounds to the sulfur economy of the water column is illustrated in Fig. 9.

The total amount of inorganic sulfur in the water column was compared with the amount predicted from dilution of seawater (Table 12). On 7/2/69, there was an enrichment of the water column with inorganic sulfur compounds. This may have been due to a diffusion of sulfide from the sediments. According to Hutchinson (1957) this is likely only in the absence of an accumulation of ferrous iron in the hypolimnion. If ferrous iron were present, the sulfide would precipitate as FeS and sediment out of the water column. It is reasonable to speculate that ferrous iron accumulation had not yet occurred by 7/2/69 in the newly formed hypolimnion. It is significant that the amount of inorganic sulfur compounds in the hypolimnion on 7/2/69 approximately equalled the amount predicted although the metalimnion was enriched in total inorganic sulfur compounds (Table 12). This is consistent with a steady state hypolimnetic sulfide content, with sulfide diffusion from the sediment to the hypolimnion balanced by sulfide diffusion from the hypolimnion to the metalimnion, where oxidation occurred.

On 7/14/69 and 8/1/69 the water column was depleted in total inorganic sulfur compounds (Table 12). Such a depletion may be explained by: 1. uptake by organisms; 2. volatilization of H_2S into the atmosphere; 3. flush of water rich in sulfur compounds out of the pond; 4. net deposition of sulfides from the water column.

The total primary productivity in the water column was estimated to be 2180 mg C/m^2/day during the summer (p. 143). This is equivalent to a demand of 54.8 mg S/m^2/day coincident with the "de novo" formation of organic matter in the water column. The standing drop of organic matter at any time is undoubtedly greater than this, but since all the trophic levels of the pond ultimately depend on primary productivity, it is unreasonable to expect a net uptake of sulfur into organic matter very much greater than 54.8 mg S/m^2/day. By this rate, it would take 967 days to form the depletion of 53,005 mg inorganic sulfur observed on 7/14/69 (Table 12); this is much longer than the residence time for the water in Oyster Pond.

According to Eriksson (1961), volatilization of hydrogen sulfide into the atmosphere from aquatic and sedimentary environments is extremely unlikely if a layer of oxygenated water is above the sulfide zone. Östlund and Alexander (1963), working with Florida Bay sediments and water and with model systems, concluded that except in very shallow areas it is doubtful that hydrogen sulfide escapes into the atmosphere, except during overturns. Overturns occasionally occur in the spring and fall in Oyster Pond, and local residents attest that hydrogen sulfide enters the atmosphere at these times (Emery, personal communication), but escape into the atmosphere is unlikely during the summer stratification.

The metalimnion, containing lighter water than the hypolimnion, was more readily exchanged than the hypolimnion, although less readily exchanged than the epilimnion. The surface currents in the pond on a typical day flowed away from the outlet due to wind-drag (Emery, 1969). This might have the effect of drawing up water from a greater depth for flow from the outlet. In this manner, it is conceivable that some metalimnion water, rich in inorganic sulfur compounds, mixed with the surface water near the outlet and flowed out of the pond.

According to Hutchinson (1957), progressive accumulation of hypolimnia with ferrous iron may be expected. The source of the iron may be largely suspended ferric hydrates and other minerals, which are abiotically reduced in the low oxidation-reduction potential water of the hypolimnion and sediments (Koyama and Sugawara, 1953). On 7/14/69, 8/1/69, and 8/15/69, the hypolimnion of Oyster Pond was depleted in inorganic sulfur compounds (Table 12). Since the enrichment in the metalimnion did not balance with the hypolimnetic depletion, deposition of sulfide minerals, primarily iron sulfides, is suggested. Net deposition of sulfides from the water column of lakes with anoxic hypolimnia is a common occurrence (Hutchinson, 1957).

The sediments had the blackened color characteristic of sediments rich in iron sulfides, and the presence of free sulfides, acid-soluble sulfides, and pyrite was confirmed by chemical analyses (Table 48). That the total amount of sulfides observed is not explicable by "in situ" sulfate-reduction is evidenced by the large excess of inorganic sulfur in the sediment compared to diluted seawater. The mean excess inorganic sulfur in the sediment was 2049 ppm (Table 48). Emery (1969) estimated the sedimentation rate of Oyster Pond to be 100 $mg/cm^2/yr$. The annual excess was thus 2049 mg S/m^2, which may have been deposited by "in situ" non-specific sulfate-reduction or by sedimentation of sulfide minerals from the overlying water column.

Nriagu (1968) estimated that 45% of the sulfides in the sediments of Lake Mendota resulted from non-specific sulfate-reduction, whereas Kaplan, et al. (1963) concluded that non-specific sulfate-reduction accounted for only a small portion of the sulfides in the California basin sediments. Lake Mendota differs from the California basins by containing greater quantities or organic matter and less sulfate. Oyster Pond contains abundant organic matter and a good supply of sulfate, although less than marine waters; on the basis of this comparison the contribution of the non-specific sulfate-reducing organisms may be expected to be intermediate between that in Lake Mendota sediments and that in the sediments of the California basins.

The mean organic matter content of the Oyster Pond sediment was 15.2% (Table 48). If this organic matter had the composition of "average organic matter" it would be 0.88% sulfur (p. 12), representing 1350 ppm sulfur. However, according to Hülsemann and Emery, the carbon:nitrogen ratio of organic matter, at station 18 in the southern basin was 9.2 (Emery, 1969). The carbon:nitrogen ratio of the model for average organic matter is 5.7, implying that the protein and amino acid fraction in the sediment was relatively low, possibly due to the presence of cellulose-containing plant residue. Since most of the organic sulfur was expected to be in the form of amino acids and protein, the sediment was estimated to contain 840 ppm organic sulfur on the basis of the organic nitrogen content. Even if 100% of the organic sulfur were released as sulfide, there would not be enough to account for the observed excess inorganic sulfur. Since Degens and Emery reported the presence of cysteine and methionine as free amino acids in the sediments of station 18, it is clear that complete decomposition of sulfur-containing organic matter in the sediment had not occurred (Emery, 1969). Considering the observed inorganic sulfur depletion in the water column, it is reasonable to conclude that deposition of sulfides from the water column is the primary source of the excess inorganic sulfur in the sediment. The question could be settled with more certainty by the use of S^{35}-labelled sulfate and organic matter.

Free sulfides, acid-soluble sulfides, and pyrite were all found in the sediment; of the three forms, free sulfides were the least abundant and pyrite by far the most abundant. The quantity of pyrite in the sediment was similar to that in the sediment of Little Round Lake, Ontario (Vallentyne, 1963). The relatively high amount of acid-soluble sulfides and pyrite may reflect an abundance of iron in the sediment of Oyster Pond (Emery and Rittenberg, 1952). The data does not lend support to the theory that black muds are being deposited too rapidly for alteration of melnikovite to pyrite to occur (Emery and Rittenberg, 1952).

The accumulation of carbonates (Tables 31-39), ammonium (Fig. 11-13), and phosphate (Fig. 14) in the hypolimnion of Oyster Pond is indicative of heterotrophic decomposition of organic matter in the water column. The data were compared to the model for complete oxidation of organic matter at the expense of dissimilatory sulfate-reduction which was proposed by Richards, et al. (1965); the model was adapted somwhat by including organic sulfur in the empirical formula for average organic matter (p. 167).

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)\ (H_2S) + 53\ SO_4^{=} \longrightarrow$$
$$106\ CO_2 + 54\ S^{=} + 16\ NH_3 + 106\ H_2O + H_3PO_4 + 2\ H^+$$

The mean ratio of oxidation equivalents (calculated by multiplying the sulfide concentration, meq/liter, times 4) to $PO_4^{\equiv}$, meq/liter, at 6.0 and 6.5 m on 7/2/69, 7/14/69, and 8/1/69 (Table 6 and Fig. 14) was 112.4, whereas the ratio predicted from the model was 216. When sulfate-depletion rather than sulfide-accumulation was used to calculate oxidation equivalents, the ratio was 243.4. Thus, the agreement with the model was considerably closer when sulfate-depletion was used than when sulfide-accumulation was used, indicating that depletion of the sulfide in the hypolimnion had occurred.

Aerobic heterotrophic bacteria were most abundant in the epilimnion whereas anaerobic heterotrophic bacteria were more abundant in the hypolimnion (Tables 25-27). Colony counts for heterotrophic bacteria were in the range of 10^3-10^4/ml during the summer. According to Jannasch and Jones (1959), the macrocolony plating procedure for enumeration of marine heterotrophic bacteria yields an underestimate of the number of heterotrophic bacteria present in the sample. They found that an MPN procedure gave counts 20 times higher than the macrocolony counts, and that a microcolony count using membrane filters gave counts 35 times higher than the macrocolony counts. Direct microscopic counts were 13-9700 times higher with seawater samples than cultural techniques. Therefore, the plate count data in Tables 25-27 were too low at least by a factor of 10.

The media used for the dissimilatory sulfate-reducing bacteria and the non-specific sulfate-reducing bacteria were quite specific (Table 30). The results of enumeration of the two groups of bacteria (Tables 28 and 29) indicated that the dissimilatory sulfate-reducing bacteria were as abundant or more abundant than the non-specific sulfate-reducing bacteria in the hypolimnion and considerably more abundant in the sediment. Considering that the dissimilatory sulfate-reducing bacteria probably produce more sulfide per cell than the non-specific sulfate-reducing bacteria, and considering the chemical evidence, it is reasonable to conclude dissimilatory sulfate-reduction is the primary source of sulfide in Oyster Pond.

An enrichment for the thiobacilli, especially _T. thioparus_, did occur near the top of the sulfide zone. However, the magnitude of this enrichment was not nearly as great as expected (Tables 13-16). Oyster Pond exhibited an abrupt transition between a zone containing reduced sulfur compounds and a zone containing oxygen or nitrate. Thus a zone containing both the required electron donors and electron acceptors for thiobacilli was not observed. The thiobacilli were most abundant in the upper part of the sulfide zone, where oxygen or nitrate may have been limiting; traces of oxygen may have been present as a result of mixing with water from above. Although *T. thioparus* may be a microaerophile (Kuznetsov and Sokolova, 1960), the organism requires oxygen. Also, the predominant reduced sulfur species in this zone were sulfide and elemental sulfur, and only traces of soluble intermediate species were present. Although *T. thioparus* is capable of growth on elemental sulfur and sulfide (Sokolova and Karavaiko, 1968), these forms are not typically considered to be the preferred substrates for the organism (Vishniac and Santer, 1957). Substrate preference by the thiobacilli in nature is a subject which warrants further study, especially with S^{35}-labelled substrates. The zonation in the Black Sea is more gradual and a broad zone between 180-210 m in the central part of the Black Sea may contain both reduced sulfur compounds and oxygen (Sorokin, 1964a; Skopintsev, *et al.*, 1964). Such a zone may be more favorable for the development of thiobacilli than the metalimnion of Oyster Pond.

A massive bloom of *Chlorobium* occurred near the top of the sulfide zone during the summer. The bloom was present at about the same depth that the *Thiobacillus* enrichment occurred. The number of *Chlorobium* cells/ml was great enough to account for most of the observed photosynthetic productivity and sulfide oxidation in the sulfide zone. Although this flora fixed a major portion of the CO_2 fixed photosynthetically in the water column during the summer, this is not truly primary productivity and such a sulfuretum cannot be self-supporting (van Gemerden, 1967). The electron donors utilized by the photosynthetic bacteria are ultimately derived from reductions occurring in the epilimnion as the result of green plant photosynthesis.

The enrichment of thiobacilli in Oyster Pond was insufficient to account for the observed dark CO_2 fixation and dark biological sulfide oxidation. This illustrates a problem inherent in ecological research. Other investigators have inferred the activity of thiobacilli in an ecosystem merely by observing their presence, or have inferred their presence by observing one of their reputed activities. Lyalikova (1957) removed Chromatium cells from lake water by filtration with a 2-3 μ membrane filter and observed that dark CO_2 fixation in the filtered water was not substantially lower than in unfiltered water; he then concluded that this was due to "chemosynthesis of thionic bacteria" which are smaller than Chromatium and capable of passing through the filter. The possibilities of dark CO_2 fixation by other chemolithotrophic bacteria or by heterotrophic organisms had not been eliminated. Ivanov (1957) observed dark biological oxidation of sulfide to elemental sulfur in the Sergiev mineral waters and observed that T. thioparus was present in these waters; he concluded: "In Sergiev Mineral Waters the most important role in the biogenic formation of sulfur is taken by thionic acid bacteria of the type of Thiobacillus thioparus." Ivanov made this conclusion in spite of the fact that he did not determine whether the population of *T. thioparus* was sufficient to account for the observed rate of sulfide oxidation to elemental sulfur.

Among the questions which remain to be answered is which other organisms play a dominant role in the dark fixation of CO_2 in the metalimnion of Oyster Pond. Evidence for the presence of several types of organisms capable of CO_2 fixation was obtained.

Other chemolithotrophic bacteria were observed using enrichment cultures (Table 24) and evidence for CO_2 fixation by methanol-oxidizing bacteria was obtained (Table 40). The substrates of other chemolithotrophic bacteria, methane, ferrous iron, ammonium, etc., certainly were present. The possibility of the participation of other chemolithotrophic bacteria in the observed dark CO_2 fixation thus remains strong. Also, the possibility that other colorless sulfur bacteria are active in CO_2 fixation should not be overlooked. Positive enrichment cultures for *Beggiatoa*, which may function as a chemolithotroph (Pringsheim and Kowallik, 1964), were obtained from Oyster Pond during the winter; this organism may be more prevalent during the summer, and such colorless sulfur bacteria as *Thiovulum* may be present as well. A cell of *Thiovulum* or *Beggiatoa* contains considerably more carbon than a *Thiobacillus* cell; consequently, a flora of these larger organisms might account for a considerable magnitude of CO_2 fixation while growing chemolithotrophically.

Ruttner (1966) noted that the occurrence of _Chromatium_ and other photosynthetic sulfur bacteria at depths in lakes below the compensation depth for other organisms suggests the possibility that they might be capable of functioning, at least partially, as chemolithotrophs. This possibility was investigated by Larsen (1953) with _Chlorobium_; he concluded that this organism was incapable of chemolithotrophic growth. "Radiosynthetic" growth at the expense of energy released during the decay of radioactive isotopes has been mentioned as a possible explanation for the occurrence of Thiorhodaceae at great depths of the Black Sea (Kriss, 1963). Although the occurrence of photosynthetic microorganisms below the depth of light penetration is difficult to explain, the "radiosynthetic" hypothesis has received little support (Sorokin, 1964a).

Dark uptake of CO_2 by _Chlorobium_ and other photosynthetic organisms has been observed (Brown, _et al._, 1947; Larsen, 1953). Such uptake, without net change in cellular carbon content, has been explained by "isotope exchange" (Larsen, 1953),

$$C^{14}O_2 + (C^{12}H_2O) \rightleftharpoons C^{12}O_2 + (C^{14}H_2O).$$

Although such reactions may occur, their quantitative significance in nature is unknown.

Heterotrophic micro- and macro-organisms are capable of fixing CO_2 (Wood ahd Stjernholm, 1962). In order to estimate whether heterotrophic bacteria could account for a substantial portion of the observed dark CO_2 fixation, it was assumed: 1. an average heterotrophic cell is a sphere 1.0 μ in diameter; 2. the cell has a specific gravity of 1.1 and 75% of the wet weight is water; 3. the dry weight is 10% ash and the organic material has the composition: $(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)$; 4. the cell is dividing logarithmically with a generation time of 2 hr; 5. 5% of this cell's carbon is derived from fixation of CO_2 (Romanenko, 1964b). Thus, starting with one cell, 3.7 x 10^{-10} mg C/hr would be fixed. If the total heterotrophic population were 10^5/ml, 37 mg C/m^3/hr would be fixed. Maximal dark CO_2 fixation during July and August ranged from 18.3-26.2 mg C/m^3/hr. Clearly, even without considering eucaryotic heterotrophic organisms, it is possible that much of the observed dark CO_2 fixation could be due to heterotrophic activity. This conclusion is supported by the stimulation of CO_2 fixation by the microflora in the presence of glucose (Table 40).

Wheatland (1954) compared rates of sulfide oxidation in vigorously shaken sterile and non-sterile flasks of Thames estuary water; he concluded that most of the oxidation in this polluted environment was due to strictly chemical reactions. The experimental design used by Wheatland was extremely artificial; such vigorous agitation with air could only be expected in storms. Ivanov (1957, 1959, 1968) used S^{35}-labelled sulfide to estimate the relative rates of biological and non-biological sulfide oxidation in waters of various types. Of the aquatic environments he studied, Lake

Belovod is the most comparable to Oyster Pond (Lyakilova, 1957). In this lake there is a well-defined layer at 13-14 m with a massive bloom of Chromatium. In this intermediate zone, abiotic oxidation of sulfide was as important quantitatively as biological sulfide oxidation; the dark biological oxidation rate was 37.5% of the abiotic rate and the photosynthetic rate was 62.3% of the abiotic rate. In the sulfide zone at 20 m, very little oxidation of sulfide was detected.

Unfortunately, Ivanov did not report the amount of labelled sulfur which appeared in the "soluble fraction" which would include sulfite, polythionates, and thiosulfate. Such intermediate sulfur species were shown by Avrahami and Golding (1968), and Cline and Richards (1969), to be very important in the pathways of non-biological oxidation of sulfide.

This investigation has demonstrated that photosynthetic and non-photosynthetic biological oxidation of sulfide at 5-6 m in the southern basin of Oyster Pond each amounted to less than 5% of the abiotic oxidation rate. Because elemental sulfur and soluble intermediate sulfur compounds were also oxidized by organisms, the rates of biological formation of sulfate were typically higher than the rates of biological sulfide oxidation.

The scheme of abiotic sulfide oxidation proposed by Avrahami and Golding (1968) (p. 25) suggests that in the presence of abundant oxygen and low sulfide concentrations, sulfate is formed by the oxidation of sulfite. Consequently, in the S^{35}-sulfide oxidation studies, the sulfate fraction would be large and the soluble fraction would be small. This model further suggests that in the presence of both oxygen and sulfide, sulfite reacts to form thiosulfate which is slowly oxidized to sulfate and elemental sulfur. Under these conditions, thiosulfate accumulates. In the presence of extremely low concentrations of oxygen, sulfite accumulates.

The data for oxidation in the presence of formalin indicated that at progressively greater depths in the water column, in the presence of progressively lower concentrations of dissolved oxygen and higher concentrations of sulfide, the soluble fraction, containing thiosulfate and sulfite, was larger and the sulfate fraction was smaller. The data clearly were in substantial agreement with the model proposed by Avrahami and Golding (1968).

Since the number of thiobacilli observed at the top of the sulfide zone was insufficient to explain the observed rates of non-photosynthetic biological oxidation of sulfide, the activity of other non-light-dependent organisms is suggested.

The presence of _Sphaerotilus_ and _Beggiatoa_ in Oyster Pond was established by the use of enrichment cultures. Both of these microorganisms are capable of depositing elemental sulfur when growing in the presence of sulfide. The occurrence of other colorless sulfur bacteria, such as _Thiovulum_, _Thiothrix_, and _Thiodendron_, in the metalimnion of Oyster Pond remains as a possibility. Rodina (1963) demonstrated the occurrence of Thiothrix and Beggiatoa in the water columns of several lakes. Lackey (1960) observed a variety of forms of colorless sulfur bacteria in a number of aquatic environments. Such organisms might be expected to develop in such environments as the metalimnion of Oyster Pond, where both sulfide and oxygen may be present (Egounov, 1898).

Heterotrophic thiosulfate-oxidizing bacteria and yeasts were also demonstrated in Oyster Pond. As much as 3% of the aerobic heterotrophic flora may have been capable of thiosulfate oxidation; this new equivalent to at least 3×10^2 thiosulfate-oxidizing heterotrophic microorganisms/ml. Heterotrophic oxidation of sulfide, elemental sulfur, tetrathionate, and sulfite by eucaryotic and procaryotic organisms has also been reported. The actual significance of heterotrophic oxidation of inorganic sulfur compounds in natural environments is a subject in need of further study.

The physical and chemical stratification of the waters of Oyster Pond provide an environment characterized by a number of vertical gradients. The vertical stratification of inorganic sulfur compounds is the indirect result of the high biological productivity of the pond and, more directly, largely the result of dissimilatory sulfate-reduction. Metallic sulfides precipitate from the water column during part of the summer, whereas at other times, there is a net enrichment of the water column with sulfide as a result of diffusion of hydrogen sulfide from the sediment.

Although most of the oxidation of sulfide in the water column is non-biological, the environment contains a diverse microflora capable of oxidizing inorganic sulfur compounds. Chlorobium is the dominant genus of photosynthetic sulfur bacteria. In terms of total numbers, CO_2 fixation, and sulfide oxidation, thiobacilli appear to play a relatively minor role in the ecology of Oyster Pond.

Table 1.

Secchi disc readings (D) in meters, computed extinction coefficients (k), and 1% light level in meters, for the southern basin in 1968.

Date	D, Meters	k	1% Light Level, Meters
7/19/68	1.4	1.21	3.8
7/23/68	1.9	0.90	5.1
7/25/68	1.9	0.90	5.1
7/29/68	1.7	1.00	4.6
8/25/68	1.0	1.70	2.7
9/8/68	1.3	1.31	3.5
9/21/68	1.6	1.06	4.3
9/22/68	1.3	1.31	3.5
1968 Mean	1.5	1.13	4.1

Table 1. Continued

Secchi disc - 1969

Date	D, Meters	k	1% Light Level, Meters
3/26/69	1.6	1.06	4.3
3/27/69	1.7	1.00	4.6
4/21/69	1.7	1.00	4.6
4/22/69	1.4	1.21	3.8
4/23/69	1.6	1.06	4.3
5/21/69	1.1	1.55	3.0
5/22/69	1.0	1.70	2.7
6/2/69	1.1	1.55	3.0
6/24/69	1.1	1.55	3.0
6/25/69	1.1	1.55	3.0
6/26/69	1.2	1.42	3.2
7/2/69	2.0	0.85	5.4
7/5/69	1.8	0.94	4.9
7/7/69	1.7	1.00	4.6
7/14/69	1.7	1.00	4.6
7/15/69	1.7	1.00	4.6

Table 1. Continued

Secchi disc - 1969

Date	D, Meters	k	1% Light Level, Meters
7/16/69	1.6	1.06	4.3
7/18/69	1.9	0.89	5.2
7/19/69	1.8	0.94	4.9
7/27/69	1.7	1.00	4.6
7/20/69	1.6	1.06	4.3
7/31/69	1.6	1.06	4.3
8/1/69	1.6	1.06	4.3
8/13/69	1.6	1.06	4.3
8/14/69	1.6	1.06	4.3
8/14/69	1.6	1.06	4.3
8/15/69	1.6	1.06	4.3
8/24/69	0.9	1.89	2.4
8/25/69	1.0	1.70	2.7
11/7/69	1.7	1.00	4.6
11/8/69	1.5	1.13	4.1
11/13/69	1.3	1.31	3.5
1969 Mean	1.5	1.13	4.1

Table 2.

Temperature readings, C, at various depths of the southern basin in 1968.

Depth, meters	Sampling Dates 7/19/68	7/23/68	7/26/68	7/29/68
0.0	25.8			
0.5		26.2	26.2	
1.0	25.8	26.2	26.2	
1.5		26.0	26.2	25.6
2.0	25.8	26.0	26.0	
2.5		26.0	25.6	
3.0	23.8	25.4	25.6	25.0
3.5		25.4	25.2	24.7
3.7	22.7			
3.9	22.3			
4.0	22.0	24.3	24.4	23.7
4.1	21.9			
4.3	21.6			
4.5	20.8	22.0	21.8	22.0
5.0	20.1	20.2	20.5	2.07
5.5		19.2	19.8	
6.0	19.0	18.6	18.7	
6.2	18.9			
6.5	18.2			

Table 2. Continued

Temperature, C - 1968

Depth, meters	Sampling Dates 8/25/68	9/8/68	9/21/68	9/22/68
0.0	22.8	21.8	21.2	20.4
0.5	22.7	21.3	20.9	
1.0	22.7	21.3	20.6	20.2
1.5	23.0	21.4	20.1	
2.0	22.6	21.4	19.9	20.1
2.5	22.4	21.8	19.5	
3.0	22.4	21.4	19.2	19.8
3.5	22.5	21.2	19.2	
4.0	22.4	21.0	19.0	
5.0	22.2	20.8	19.0	19.0
5.3			18.9	
5.5	21.0	20.4	18.7	19.0
5.7			18.7	18.9
6.0	19.4	19.6	18.7	18.7
6.2			18.7	18.7
6.5	18.5	18.7	18.5	18.5

Table 2. Continued

Temperature, C - 1969

Depth, meters	Sampling Dates 3/26/69	4/21/69	5/21/69	6/2/69
0.0	7.0	12.0	17.7	19.3
0.5	6.8	11.8	17.7	19.3
1.0	6.8	11.8	17.4	19.3
1.5	6.8	11.8	17.3	10.2
2.0	6.8	11.7	17.2	19.1
2.5	6.8	11.7	17.2	19.0
3.0	6.7	11.8	17.2	18.0
3.5	6.7	11.6	17.2	18.0
4.0	6.7	11.4	17.0	18.0
4.5	6.8	11.4	17.2	18.1
5.0	6.7	11.0	17.2	18.2
5.5	6.6	11.1	17.1	18.3
6.0	6.6	11.0	17.2	18.5
6.5	6.4	11.0	17.2	18.7

Table 2. Continued

Temperature, C - 1969

Depth, meters	Sampling Dates 6/24/69	7/2/69	7/5/69	7/7/69
0.0	21.1	24.2	23.5	22.8
0.5	20.9	23.9	23.2	22.6
1.0	20.6	23.9	23.1	22.8
1.5	20.5	23.9	23.1	22.8
2.0	20.4	23.3	22.9	22.4
2.5	20.3	23.0	22.8	22.7
3.0	20.3	22.7	22.7	22.5
3.5	20.3	21.9	22.6	22.7
4.0	20.3	21.3	21.9	22.0
4.5	20.5	21.7	21.4	21.0
5.0	20.2	21.1	21.0	20.5
5.5	19.9	20.1	20.0	19.8
6.0	18.1	19.3	19.3	18.7
6.5	17.6	18.9	19.0	17.9

Table 2. Continued

Temperature, C - 1969

Depth, meters	Sampling Dates and Times			
	7/14/69	7/18/69	7/27/69	7/31/69 2400 hr
0.0	21.8	25.5	21.4	24.2
0.5	21.7	25.7	21.2	24.0
1.0	22.1	25.4	21.2	23.8
1.5	21.8	25.0	21.2	24.0
2.0	21.7	24.4	21.2	24.0
2.5	21.7	24.0	21.2	23.9
3.0	21.5	23.9	21.0	23.7
3.5	21.3	23.2	21.0	22.4
4.0	21.2	22.4	21.0	22.3
4.3				22.0
4.5	21.0	22.0	20.8	21.1
4.7				21.1
5.0	20.8	21.6	20.2	20.9
5.5	20.2	20.5	20.0	20.4
6.0	19.7	20.3	19.7	20.3
6.5	19.7	19.8	17.9	20.3

Table 2. Continued

Temperature, C - 1969

Depth, meters	Sampling Dates and Times			
	8/1/69 1200 hr	8/15/69	8/24/69	11/7/69
0.0	24.9	26.2	25.0	10.6
0.5	24.9	25.7	25.2	10.4
1.0	24.7	25.5	24.7	10.4
1.5	24.4	25.3	24.0	10.4
2.0	24.3	25.0	23.7	10.4
2.5	23.9	25.0	23.4	10.4
3.0	23.4	24.8	23.1	10.4
3.5	23.2	24.7	23.0	10.4
4.0	22.4	24.5	23.0	10.4
4.3	22.3			
4.5	31.9	23.3	22.9	10.4
4.7	21.2			
5.0	21.0	21.7	22.5	10.4
5.5	21.0	20.6	21.9	10.5
6.0	20.5	20.0	20.6	10.5
6.5	20.2	19.4	19.7	11.7

Table 3.

Chlorinity determinations, ppt, at various depths of the southern basin in 1968.

Depth, Meters	Sampling Dates 7/19/68	7/23/68	7/25/68	7/29/68	8/25/68
0.0	0.73				0.79
0.5		0.73	0.68		0.78
1.0	0.76	0.70	0.73	0.77	
1.5		0.80	0.74	0.71	0.77
2.0	0.76	0.74	0.73		0.77
2.5		0.73	0.73		0.78
3.0	0.79	0.71	0.76	0.78	0.78
3.5		0.73	0.71	0.76	0.80
4.0	0.89	0.76	0.78	0.82	0.82
4.5	0.95	0.86	0.91	0.79	0.88
5.0	1.03	1.14	1.10	1.09	0.96
5.5		1.46	1.38		2.00
6.0	1.95	2.05	1.94		2.18
6.5	2.29				2.47

Table 3. Continued

Chlorinity, ppt - 1968-69

Depth, meters	Sampling Dates 9/8/68	9/22/68	3/26/69	4/21/69	5/21/69
0.0	0.71	0.68	0.77	0.68	0.74
0.5	0.76	0.79	0.83	0.74	0.68
1.0	0.76	0.81	0.77	0.78	0.83
1.5	0.80	0.82	0.76	0.69	0.70
2.0	0.76	0.81	0.77	0.72	0.72
2.5	0.76	0.78	0.78	0.73	0.66
3.0	0.78	0.79	0.75	0.72	0.67
3.5	0.76	0.77	0.80		0.70
4.0	0.78	0.78	0.76	0.71	0.70
4.5	0.78	0.77	0.92	0.82	0.69
5.0	0.85	0.80	0.72	0.68	0.71
5.5	1.56	0.81	0.88	0.76	0.71
6.0	2.17	0.81	0.72	0.72	0.70
6.5	2.38	2.14	1.02	0.82	

Table 3. Continued

Chlorinity, ppt - 1969

Depth, meters	Sampling Dates 6/2/69	6/24/69	7/2/69	7/7/69	7/14/69
0.0	0.77	0.75	0.75	0.77	0.78
0.5	0.66	0.81	0.77	0.76	0.83
1.0	0.81	0.74	0.74	0.73	0.84
1.5	0.82	0.66	0.79	0.76	0.83
2.0	0.99	0.77	0.76	0.76	0.72
2.5	0.71	0.68	0.78	0.73	0.80
3.0	0.77	0.72	0.78	0.76	0.80
3.5	0.81	0.71	0.86	0.81	0.79
4.0	0.91	0.70	0.89	0.91	0.78
4.5	1.00	0.73	1.09	1.00	0.80
5.0	1.15	0.77	1.42	1.59	1.60
5.5	1.34	0.85	1.67	1.59	1.95
6.0	1.55	1.51	1.87	1.84	2.09
6.5		1.57	1.74	1.92	2.13

Table 3. Continued

Chlorinity, ppt - 1969

Depth, meters	Sampling Dates and Times				
	7/18/69	7/27/69	7/31/69		
			1900 hr	2100 hr	2400 hr
0.0	0.78	0.86			0.79
0.5	0.80	0.79			
1.0	0.80	0.78			0.81
1.5	0.80	0.80			
2.0	0.78	0.82			0.78
2.5	0.87	0.81			
3.0	0.83	0.79			0.90
3.5	0.84	0.84			
4.0	0.79		1.28	1.26	1.35
4.3			1.58	1.61	1.62
4.5	0.88	1.33	1.76		1.69
4.7			2.10	2.08	2.15
5.0	1.59	2.01	2.36	2.33	2.40
5.5	1.86	2.30			
6.0	2.13	2.86			3.41
6.5		2.89			3.94

Table 3. Continued

Chlorinity, ppt - 1969

Depth, meters	Sampling Dates and Times				
	8/1/69				
	0300 hr	0600 hr	0900 hr	1200 hr	1530 hr
0.0				0.76	
1.0				0.83	
2.0				0.74	
3.0				0.92	
4.0	1.36	1.37	1.28	1.28	1.32
4.3	1.67	1.69	1.63	1.63	1.59
4.5				1.78	
4.7	2.18	2.14	2.16	2.16	2.10
5.0	2.32	2.29	2.30	2.28	2.34
6.0				3.21	
6.5				3.88	

Table 3. Continued

Chlorinity, ppt - 1969

Depth, meters	Sampling Dates and Times 8/14/69 0900 hr	1100 hr	1300 hr
4.5	1.52	1.48	1.53
5.0	2.20	2.14	2.19

Depth, meters	Sampling Dates and Times 8/14/69 1500 hr	1700 hr	1900 hr
4.5	1.52	1.51	1.54
5.0	2.11	2.20	2.06

Table 3. Continued

Chlorinity, ppt - 1969

Depth, meters	Sampling Dates 8/15/69	8/24/69	11/7/69
0.0	0.84	0.84	1.03
0.5	0.82	0.81	1.04
1.0		0.86	1.02
1.5	0.79	0.88	1.07
2.0	0.79	0.81	1.02
2.5	0.88	0.83	1.04
3.0	0.81	0.83	1.13
3.5	0.82	0.83	1.02
4.0	0.84	0.88	1.02
4.5	1.48	1.24	1.00
5.0	2.35	2.38	1.06
5.5	2.79	2.66	0.97
6.0	3.26	3.22	1.02
6.5	3.64	3.03	0.96

Table 4.

pH measurements at various depths of the southern basin in 1968

Depth, meters	Sampling Dates 7/23/68	7/25/68	7/29/68	8/25/68
0.0				7.42
0.5	8.45	8.37		8.23
1.0	8.37	8.40		8.15
1.5	8.55	8.52	8.18	8.13
2.0	8.50	8.43		8.31
2.5	8.50	8.47		8.22
3.0	8.32	8.25	8.20	8.03
3.5	7.72	7.27	8.03	8.42
4.0	6.80	6.78	7.29	8.27
4.5	6.72	6.70	6.98	7.55
5.0	6.68	6.87	6.70	6.95
5.5	6.68	6.94		6.81
6.0	6.70	6.98		6.80
6.5				6.75

Table 4. Continued

pH - 1968-69

Depth, meters	Sampling Dates 9/8/68	9/21/68	9/22/68	3/26/69
0.0	7.19	9.87	9.68	6.87
0.5	7.51	9.90		7.37
1.0	7.48	9.94	9.69	7.37
1.5	7.52	9.90		7.39
2.0	7.52	9.87	9.62	7.34
2.5	7.58	9.62		7.32
3.0	7.56	9.15	9.33	7.30
3.5	7.57	8.37		7.26
4.0	7.59	8.39	8.82	7.22
4.5	7.53	8.21		7.19
5.0	7.32	7.80	7.52	7.26
5.3		7.47		
5.5	6.98	7.18	6.64	7.22
5.7		7.13	6.64	
6.0	6.98	6.83	6.50	7.25
6.2		6.75	6.52	
6.5	7.02	6.70	6.47	7.07

Table 4. Continued

pH - 1969

Depth, meters	Sampling Dates			
	4/21/69	5/21/69	6/2/69	6/24/69
0.0	7.13	7.10	7.73	7.87
0.5	7.28	7.37	7.80	7.91
1.0	7.36	7.22	7.78	7.70
1.5	7.39	7.38	7.73	7.52
2.0	7.39	7.16	7.69	7.48
2.5	7.38	7.38	7.62	7.42
3.0	7.42	7.37	7.08	7.39
3.5	7.40	7.32	6.96	7.45
4.0	7.40	7.32	7.00	7.45
4.5	7.38	7.35	6.92	7.34
5.0	7.31	7.37	6.90	7.31
5.5	7.27	7.37	6.84	6.82
6.0	7.24	7.35	6.89	6.86
6.5	7.04	6.68	6.87	6.70

Table 4. Continued

pH - 1969

Depth, meters	Sampling Dates 7/7/69	7/14/69	7/18/69	7/27/69
0.0	8.76	7.54	8.14	7.61
0.5	8.81	7.53	8.40	7.67
1.0	8.81	7.60	8.43	7.47
1.5	8.83	7.62	8.47	7.87
2.0	8.87	7.64	8.10	7.85
2.5	8.86	7.60	7.75	7.85
3.0	8.81	7.63	7.64	7.64
3.5	8.87	7.63	7.40	7.47
4.0	7.61	7.63	6.90	6.99
4.5	6.99	7.60	6.48	6.87
5.0	6.89	6.82	6.80	6.89
5.5	6.92	6.84	6.80	6.88
6.0	6.92	6.84	6.75	6.92
6.5	6.91	6.80	6.68	6.92

Table 4. Continued

pH - 1969

Depth, meters	Sampling Dates and Times				
	7/31/69 2400 hr	8/1/69 1200 hr	8/15/69	8/24/69	11/7/69
0.0	8.78	8.80	9.10	9.82	7.32
0.5	8.80	8.78	9.22	9.83	7.41
1.0	8.73	8.83	9.20	9.72	7.32
1.5	8.78	8.87	9.23	9.60	7.33
2.0	8.73	8.60	9.09	9.24	7.37
2.5	8.27	7.88	8.87	9.04	7.40
3.0	7.93	7.43	8.14	8.60	7.38
3.5	7.38	7.22	7.66	8.55	7.38
4.0	6.78	6.74	7.17	8.40	7.38
4.3	6.90	6.82			
4.5	6.78	6.90	6.88	7.39	7.38
4.7	6.83	6.83			
5.0	6.79	6.84	6.92	6.73	7.40
5.5	6.82	6.77	6.89	6.81	7.38
6.0	6.84	6.78	6.91	6.81	7.37
6.5	6.81	6.80	6.93	6.85	6.94

Table 5.

Dissolved oxygen determinations, ppm, at various depths of the southern basin in 1968.

Depth, meters	Sampling Dates 7/23/68	7/25/68	7/29/68	8/25/68	9/8/68	9/22/68
0.0				8.33	9.05	11.95
0.5	9.13					
1.0	8.70			8.53	9.22	12.05
1.5		8.66	8.31			
2.0	8.55			8.65	8.77	11.92
3.0	8.25	7.80	7.86	8.77	8.97	10.69
3.5			5.31			
4.0	3.03	1.97	2.43	8.27	9.02	8.90
4.5	0.00	0.00	0.00	7.12	9.23	
5.0				2.83	7.50	7.39
5.5				0.00	0.00	4.52
6.0						0.81

Table 5. Continued

Dissolved oxygen, ppm - 1969

Depth, meters	Sampling Dates 3/26/69	4/21/69	5/21/69	6/2/69	6/24/69
0.0	8.57	10.72	10.13	9.72	9.21
1.0	9.10	10.50	10.04	9.81	8.91
2.0	9.02	10.18	9.95	10.02	8.81
3.0	8.60	10.48	10.13	6.81	8.75
4.0	8.00	10.56	9.92	7.22	8.55
5.0	6.66	10.72	10.31	6.51	8.06
5.5					4.11
6.0	6.62	10.78	10.51	6.07	0.00
6.5	4.69	5.95	2.99	4.88	

Table 5. Continued

Dissolved oxygen, ppm - 1969

Depth, meters	Sampling Dates				
	7/2/69	7/7/69	7/14/69	7/18/69	7/27/69
0.0	8.42	9.05	9.97	9.18	9.21
1.0	7.96	9.50	9.52	9.51	9.23
2.0	7.62	10.15	8.85	9.23	9.27
3.0	4.71	8.66	9.10	7.57	9.90
4.0	2.70	6.51	8.74	6.51	7.49
4.5	3.00	1.65	8.83	3.80	2.48
5.0	1.02	0.00	0.00	0.00	0.00
5.5	1.00	0.00	0.00		0.00
6.0	0.00				

Table 5. Continued

Dissolved oxygen, ppm - 1969

Depth, meters	Sampling Dates				
	7/31/69 2400 hr	8/1/69 1200 hr	8/15/69	8/24/69	11/7/69
0.0	9.87	9.32	10.25	8.25	9.33
1.0	9.47	10.32	10.67	9.73	9.81
2.0	9.69	9.40	9.84	9.21	10.14
3.0	8.23	6.48	7.73	7.58	10.00
3.5			6.73		
4.0	4.13	3.08	5.53	6.07	9.85
4.3	1.37	0.54	0.00		
4.5	0.00	0.00		1.12	
5.0				0.00	10.77
6.0					9.85
6.5					0.00

Table 6.

Sulfide determinations, ppm sulfide-S, at various depths of the southern basin in 1968.

Depth, meters	Sampling Dates 7/25/68	7/29/68	8/25/68	9/8/68	9/22/68
4.0	0.0	0.0	0.0	0.0	
4.5	3.5	0.0	0.0	0.0	
5.0	7.6	11.1	0.0	0.0	
5.3					0.0
5.5	16.1		23.1	14.7	0.0
5.7					0.0
6.0	33.4		37.2	26.4	3.2
6.2					15.0
6.5	46.5		50.9	47.3	38.6

Table 6. Continued

Sulfide, ppm sulfide-S - 1969

Depth, meters	Sampling Dates 3/27/69	4/21/69	5/21/69	6/2/69	6/24/69
5.0					0.0
5.5					0.0
6.0	0.0	0.0	0.0	0.0	11.8
6.5	0.0	1.3	0.83	0.95	17.2

Table 6. Continued

Sulfide, ppm sulfide-S - 1969

Depth, meters	Sampling Dates 7/2/69	7/7/69	7/14/69	7/18/69	7/27/69
4.0	0.0	0.0	0.0	0.0	0.0
4.5	0.0	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.48	0.56	3.8
5.5	4.3	0.81	0.67	12.6	20.0
6.0	9.2	8.2	9.9	14.9	26.2
6.5	12.5	13.5	13.3	16.4	24.2

Table 6. Continued

Sulfide, ppm sulfide-S - 1969

Depth, meters	Sampling Dates and Times						
	7/31/69 2400 hr	8/1/69 1200 hr	8/15/69	8/24/69	11/7/69	11/8/69	11/13/69
4.0	0.0		0.0	0.0			
4.3	0.0						
4.5	0.90	1.5	2.4	0.0			
5.0	5.3	4.0	4.7	1.8			
5.5	21.5	19.7	22.5	20.4			
6.0	28.3	26.5	25.2	24.6	0.0	0.0	
6.5	28.3	28.9	27.3	28.3	5.9	4.8	0.0

Table 7.

Elemental sulfur determinations, ppm elemental S, at various depths of the southern basin in 1968-69.

Depth, meters	Sampling Dates 7/25/68	8/25/68	7/2/69
3.5	0.0		
4.0	11.4		
4.2			
4.5	4.0	0.0	0.0
5.0	0.0	8.0	8.2
5.5		0.0	0.0

Depth, meters	Sampling Dates 7/14/69	8/1/69	8/15/69
3.5		0.0	
4.0		9.2	0.0
4.2			7.8
4.5	0.0	0.0	0.0
4.8	6.4		
5.0	4.2		
5.5	0.0		

Table 8.

Thiosulfate determinations, ppm thiosulfate-S, at various depths of the southern basin in 1968.

Depth, meters	Sampling Dates 7/25/68	7/29/68	8/25/68	9/8/68
0.5	0.0			
1.0	0.0			
1.5	0.0			
2.0	0.0			
2.5	0.0			
3.0	0.0			
3.5	0.0			
4.0	0.0		0.0	
4.5	0.0	0.0	0.0	
5.0	0.45	1.10	0.0	0.19
5.4				0.55
5.5	0.72		1.14	0.95
5.6				1.01
6.0	0.90		1.09	1.43
6.2				1.32

Table 8. Continued

Thiosulfate, ppm thiosulfate-S - 1969

Depth, meters	Sampling Dates 3/27/69	4/21/69	5/22/69	6/2/69	6/24/69
4.0	0.0	0.0	0.0	0.0	0.0
4.5	0.0	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.0	0.0	0.0
5.5	0.0	0.0	0.0	0.0	0.0
6.0	0.0	0.0	0.0	0.20	0.68

Depth, meters	Sampling Dates 7/2/68	7/14/69	7/27/69	8/15/69	8/24/69
4.0	0.0		0.0	0.0	0.0
4.5	0.0	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.20	0.35	0.44
5.5	0.22	0.41	0.49	0.66	0.83
6.0	0.78	0.71	0.77	0.94	0.86

Table 9.

Polythionate determinations, ppm tetrathionate-S, at various depths of the southern basin in 1968.

Depth, meters	Sampling Dates 7/25/68	7/29/68	8/25/68	9/8/68
0.5	0.0			
1.0	0.0			
1.5	0.0			
2.0	0.0			
2.5	0.0			
3.0	0.0			
3.5	0.0			
4.0	0.0		0.0	
4.5	0.0	0.0	0.0	
5.0	0.0	0.0	0.0	0.0
5.4				0.35
5.5	0.0		1.49	0.57
5.6				0.86
6.0	0.28		1.37	0.88
6.2				0.92

Table 9. Continued

Polythionates, ppm tetrathionate-S - 1969

Depth, meters	Sampling Dates 3/27/69	4/21/69	5/22/69	6/2/69	6/24/69
4.0	0.0	0.0	0.0	0.0	0.0
4.5	0.0	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.0	0.0	0.0
5.5	0.0	0.0	0.0	0.0	0.0
6.0	0.0	0.0	0.0	0.0	0.30

Depth, meters	Sampling Dates 7/2/69	7/14/69	7/27/69	8/15/69	8/24/69
4.0	0.0		0.0	0.0	0.0
4.5	0.0	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.0	0.0	0.0
5.5	0.0	0.0	0.0	0.59	0.63
6.0	0.30	0.38	0.45	1.01	1.03

Table 10.

Sulfite determinations, ppm sulfite-S, at various depths of the southern basin in 1969.

Depth, meters	Sampling Dates 6/2/69	7/2/69	7/14/69	7/27/69	8/15/69
4.0	0.0	0.0	0.11	0.10	0.20
4.5	0.0	0.07	0.11	0.10	0.26
5.0	0.0	0.06	0.20	0.14	0.26
5.5	0.0	0.11	0.23	0.20	0.47
6.0	0.0	0.15	0.26	0.25	0.49
6.5	0.11	0.15	0.29	0.30	0.70

Table 11.

Sulfate determinations, ppm sulfate-S, at various depths of the southern basin in 1969.

Depth, meters	Sampling Dates and Times				
	4/21/69	7/2/69	7/14/69	7/31/69	
				1900 hr	2100 hr
0.0	34.0	23.8	32.0		
0.5		25.9			
1.0	33.0	36.9	37.3		
1.5		37.3			
2.0	33.3		30.5		
2.5		35.7			
3.0	33.0	40.1	39.3		
3.5		39.8			
4.0	32.3	42.8	41.3	62.0	61.5
4.3				92.5	93.0
4.5		77.2	43.3	95.0	
4.7				85.0	84.5
5.0	32.0	62.0	40.5	85.5	84.5
5.5		72.3			
6.0	22.3	67.7	62.0		
6.5		69.8	83.7		

Table 11. Continued

Sulfate, ppm sulfate-S - 1969

Depth, meters	Sampling Dates and Times				
	7/31/69	8/1/69			
	2400 hr	0300 hr	0600 hr	0900 hr	1200 hr
0.0	33.5				33.3
1.0	34.4				35.5
2.0	35.5				34.2
3.0	43.0				42.0
4.0	64.5	64.7	66.5	61.5	61.0
4.3	95.5	96.5	98.0	95.5	96.5
4.5	93.0				99.0
4.7	79.5	79.0	78.0	78.0	80.5
5.0	84.0	82.0	84.5	80.5	82.0
6.0	109.0				105.0
6.5	130.0				123.0

Table 11. Continued

Sulfate, ppm sulfate-S - 1969

Depth, meters	Sampling Dates and Times				
	8/1/69	8/14/69			
	1530 hr	0900 hr	1100 hr	1300 hr	1500 hr
4.0	64.5				
4.3	93.5				
4.5		71.0	68.5	70.3	71.0
4.7	82.0				
5.0	85.5	70.0	72.5	73.0	76.0

Depth, meters	Sampling Dates and Times		
	8/14/69		8/15/69
	1700 hr	1900 hr	
0.0			39.2
1.0			40.0
2.0			35.7
3.0			43.3
4.0			48.0
4.5	69.0	72.0	66.7
5.0	78.5	78.5	85.3
6.0			110.6
6.5			145.0

Table 12.

Inorganic sulfur balances in the southern basin on various dates. The actual concentrations (ppm S) of each inorganic sulfur species were plotted vs. depth. The area of each curve was used to compute the total amount of sulfur (mg) as that constituent in one m^3 depth intervals of the water column. The amount of actual sulfur was subtracted from the amount predicted from a seawater sulfate:chloride ratio of 0.1394 in order to determine enrichment or depletion relative to seawater.

7/2/69

Depth Interval, Meters	Predicted $SO_4^{=}$-S	Actual S							Predicted S minus Actual S
		$SO_4^{=}$	$SO_3^{=}$	$S_4O_6^{=}$	$S_2O_3^{=}$	S^o	$S^{=}$	Total Actual S	
0-1	35,000	27,900	0	0	0	0	0	27,900	+ 7,100
1-2	35,500	36,900	0	0	0	0	0	36,900	- 1,400
2-3	36,000	37,200	0	0	0	0	0	37,200	- 1,200
3-4	38,800	41,500	0	0	0	0	0	41,500	- 2,700
4-5	51,400	66,100	50	0	0	2,050	0	68,200	- 16,800
5-6	76,900	68,900	108	75	305	2,050	4,450	75,888	+ 1,012
6-6.5	41,200	34,500	75	213	465	0	6,425	41,678	- 478
Sum	314,800	313,000	233	288	770	4,100	10,875	329,266	- 14,466

Table 12. Continued

Inorganic sulfur balances - Total amount of sulfur (mg) as various inorganic species in one m^3 depth intervals vs. amount predicted from seawater sulfate.

7/14/69

Depth Interval, Meters	Predicted $SO_4^{=}$-S	Actual S: $SO_4^{=}$	$SO_3^{=}$	$S_4O_6^{=}$	$S_2O_3^{=}$	S^o	$S^{=}$	Total Actual S	Predicted S minus Actual S
0-1	38,200	34,700	0	0	0	0	0	34,700	+ 3,500
1-2	37,500	33,900	0	0	0	0	0	33,900	+ 3,600
2-3	36,300	34,900	0	0	0	0	0	34,900	+ 1,400
3-4	36,800	40,300	28	0	0	0	0	40,328	- 3,528
4-5	55,800	42,100	133	0	0	2,020	120	44,373	+ 11,427
5-6	88,600	51,300	230	95	383	420	854	53,282	+ 35,318
6-6.5	44,300	36,400	138	295	379	0	5,800	43,012	- 1,288
Sum	337,500	273,600	529	390	762	2,440	6,774	284,495	+ 53,005

Table 12. Continued

Inorganic sulfur balances - Total amount of sulfur (mg) as various inorganic species in one m^3 depth intervals vs. amount predicted from seawater sulfate.

		8/1/69							
		Actual S							
Depth Interval, Meters	Predicted $SO_4^{=}$-S	$SO_4^{=}$	$SO_3^{=}$	$S_4O_6^{=}$	$S_2O_3^{=}$	S^o	$S^{=}$	Total Actual S	Predicted S minus Actual S
0-1	37,000	34,400	0	0	0	0	0	34,400	+ 2,600
1-2	36,500	34,900	0	0	0	0	0	34,900	+ 1,600
2-3	37,600	38,100	0	0	0	0	0	38,100	- 500
3-4	51,200	51,500	25	0	0	0	0	51,525	- 325
4-5	83,700	82,000	110	0	50	2,300	1,750	86,210	- 2,510
5-6	127,500	93,500	168	113	488	2,300	17,480	114,049	+ 13,451
6-6.5	82,300	57,000	137	338	443	0	13,850	71,768	+ 10,532
Sum	455,800	391,400	440	451	981	4,600	33,080	430,952	+ 24,848

Table 12. Continued

Inorganic sulfur balances - Total amount of sulfur (mg) as various inorganic species in one m^3 depth intervals vs. amount predicted from seawater sulfate.

8/15/69

Depth Interval, Meters	Predicted $SO_4^{=}$-S	Actual S $SO_4^{=}$	$SO_3^{=}$	$S_4O_6^{=}$	$S_2O_3^{=}$	S^o	$S^{=}$	Total Actual S	Predicted S minus Actual S
0-1	38,200	39,600	0	0	0	0	0	39,600	- 1,400
1-2	36,900	37,900	0	0	0	0	0	37,900	- 1,000
2-3	39,100	39,500	0	0	0	0	0	39,500	- 400
3-4	38,300	45,700	50	0	0	0	0	45,750	- 7,450
4-5	71,400	66,700	245	0	88	1,850	2,375	71,258	+ 142
5-6	129,700	98,000	422	548	655	0	18,725	118,350	+ 11,350
6-6.5	80,000	63,800	298	653	548	0	13,125	78,424	+ 1,576
Sum	433,600	391,200	1,015	1,201	1,291	1,850	34,225	430,782	+ 2,818

Table 13.

Enumeration of Thiobacilli at various depths of the southern basin in 1968. *T. thioparus* MPN/100 ml using Medium A. *T. thiooxidans* MPN/100 ml using Medium D. *T. denitrificans* MPN/100 ml using Medium G.

Depth, meters	*T. thioparus*	*T. thiooxidans*	*T. denitrificans*
		7/29/68	
1.5	0	0	0
3.0	0	0	0
3.5	0	0	0
4.0	0	0	0
4.5	0	0	0
5.0	490	80	0
		9/8/68	
5.0	930	70	0
5.4	930	70	0
5.5	930	150	0
5.6	2400	40	90
6.0	930	40	40
6.2	230	0	0

Table 14.

Enumeration of *T. thioparus* at various depths of the southern basin in 1969. MPN/100 ml using Medium B.

Depth, meters	3/27/69	4/23/69	5/20/69	6/24/69	7/5/69	7/31/69	8/14/69	8/25/69
	Sampling Dates							
1.5						20		
4.0		0	170	20	80	40	80	50
5.0	80	130	230	110	390	1,090	4,900	2,100
5.5				130	1,100	3,300	700	80
6.0	130	330	270	800	490	70	20	50
6.5	230	790	490	80	40	0	0	

Table 15.

Enumeration of *T. thiooxidans* at various depths of the southern basin in 1969. MPN/100 ml using Medium E.

Depth, meters	3/27/69	4/23/69	5/20/69	6/24/69	7/5/69	7/31/69	8/14/69	8/25/69
	Sampling Dates							
1.5						0		
4.0		0	0				0	20
5.0	0	0	0	0	20	140	70	20
5.5				90	140	50	0	80
6.0	0	20	40	40	20	20	0	0
6.5	50	130	80	20	0			

Table 16.

Enumeration of *T. denitrificans* at various depths of the southern basin in 1969. MPN/100 ml using Medium H.

Depth, meters	Sampling Dates 3/27/69	4/23/69	5/20/69	6/24/69	7/5/69	7/31/69	8/14/69	8/25/69
1.5						0		
4.0		0	0				0	0
5.0	0	0	0	0	0		0	20
5.5				0	0	0	0	80
6.0	0	0	0	50	60	50	0	50
6.5	0	0	0	0	20	20		

Table 17.

Comparison of *T. thioparus* MPN medium and incubation used in 1968 (10 ml Medium A, Static Incubation) with those used in 1969 (5 ml Medium B, Shaken Incubation). 0, 10^2, and 10^4 cells/100 ml of a pure culture of *T. thioparus* were added to water samples from various depths of the southern basin. MPN/100 ml.

	Added *T. thioparus* cells/100 ml		
	0	10^2	10^4
		3/27/69, 5.0 m	
1968 MPN Procedure	20	80	4900
1969 MPN Procedure	80	240	9500
		3/27/69, 6.5 m	
1968 MPN Procedure	130	430	7900
1969 MPN Procedure	230	460	11000
		5/20/69, 5.0 m	
1968 MPN Procedure	130	210	4900
1969 MPN Porcedure	130	270	7900
		5/20/69, 6.5 m	
1968 MPN Procedure	640	790	7900
1969 MPN Procedure	790	840	7000

Table 18.

Comparison of *T. thioparus* MPN medium and incubation used in 1968 (10 ml Medium A, Static Incubation) with those used in 1969 (5 ml Medium B, Shaken Incubation) and with a plating procedure (Spread Plates, Medium C). 0, 10^2, 10^3, 10^4, 10^5, and 10^6 cells/100 ml of a pure culture of T. thioparus were added to water samples from various depths of the southern basin. MPN/100 ml or Plate Count/100 ml.

	Added *T. thioparus* cells/100 ml					
	0	10^2	10^3	10^4	10^5	10^6
				11/7/69, 5.5 m		
1968 MPN Procedure	0	92	1300	7900	46000	490000
1969 MPN Procedure	0	140	1300	7900	49000	700000
Plating Procedure	4000			11000		710000
				11/7/69, 6.2 m		
1968 MPN Procedure	0	140	1300	7900	79000	490000
1969 MPN Procedure	50	110	1400	11000	79000	490000
Plating Procedure	6000			15000		680000

Table 19.

Comparison of *T. thiooxidans* MPN medium and incubation used in 1968 (10 ml Medium D, Static Incubation) with those used in 1969 (5 ml Medium E, Shaken Incubation). 0, 10^2, and 10^4 cells/100 ml of a pure culture of *T. thiooxidans* were added to water samples from various depths of the southern basin. MPN/100 ml.

	Added *T. thiooxidans* cells/100 ml		
	0	10^2	10^4
		3/27/69, 5.0 m	
1968 MPN Procedure	20	130	3300
1969 MPN Procedure	0	170	4900
		3/27/69, 6.5 m	
1968 MPN Procedure	20	50	1300
1969 MPN Procedure	50	310	4600
		5/20/69, 5.0 m	
1968 MPN Procedure	0	130	3300
1969 MPN Procedure	0	130	7900
		5/20/69, 6.5 m	
1968 MPN Procedure	50	61	2300
1969 MPN Procedure	80	240	4900

Table 20.

Comparison of *T. denitrificans* MPN medium and incubation used in 1968 (10 ml Medium G) with those used in 1969 (5 ml Medium H). 0, 10^2, and 10^4 cells/100 ml of a pure culture of *T. denitrificans* were added to water samples from various depths of the southern basin. MPN/100 ml.

	Added *T. denitrificans* cells/100 ml		
	0	10^2	10^4
		3/27/69, 5.0 m	
1968 MPN Procedure	0	20	3300
1969 MPN Procedure	0	80	3500
		3/27/69, 6.5 m	
1968 MPN Procedure	0	50	2300
1969 MPN Procedure	0	50	3300
		5/20/69, 5.0 m	
1968 MPN Procedure	0	80	3500
1969 MPN Procedure	0	50	4900
		5/20/69, 6.5 m	
1968 MPN Procedure	0	50	4900
1969 MPN Procedure	0	20	3300

Table 21.

Characteristics of Stock Cultures and Isolated Strains of Thiobacilli

	T. thioparus #A	*T. thiooxidans* #B	*T. denitrificans* #C
Gram Reaction:	neg.	neg.	neg.
Cell Size:	0.6 x 1.5 μ	0.8 x 1.2 μ	0.5 x 1.5 μ
Motility:	+	+	+
Colonial Morphology:	creamy yellow raised circular	translucent raised circular	creamy yellow raised circular
Anaerobic Growth:	±	0	++++
Gas Production - aerobic:	0	0	0
anaerobic:	0	0	+
Growth at 4 C	++	±	±
20 C	++++	++	+++
28 C	++++	++++	++++
37 C	++	±	±
55 C	0	0	0

Table 21. Continued

Characteristics of Stock Cultures and Isolated Strains of Thiobacilli

	T. thioparus #A	*T. thiooxidans* #B	*T. denitrificans* #C
Growth at pH 3.0	0	++++	0
4.0	0	++++	0
5.0	0	++++	0
6.0	++	±	++
7.0	++++	0	++++
8.0	+++	0	++++
9.0	0	0	0
Growth on thiosulfate:	++++	+++	++++
final pH:	4.0	2.6	4.0
Growth on sulfur:	++	++++	+
final pH:	6.0	1.0	6.5
Growth in organic media:			
Nutrient Broth:	0	0	0
Medium R :	0	0	0

Table 21. Continued

Characteristics of Stock Cultures and Isolated Strains of Thiobacilli

	Oyster Pond Isolates			
	#MS-5	#MS-11	#MS-17	#MS-25
Gram Reaction:	neg.	neg.	neg.	neg.
Cell Size:	0.5 x 1.5 μ	0.6 x 1.2 μ	0.5 x 1.5 μ	0.6 x 1.8 μ
Motility:	+	+	+	+
Colonial Morphology:	creamy yellow raised circular	translucent raised circular	creamy yellow raised circular	creamy white raised circular
Anaerobic Growth:	±	0	++++	0
Gas Production -				
aerobic:	0	0	0	0
anaerobic:	0	0	+	0
Growth at 4 C	++	+	++	++
20 C	+++	+++	+++	++
28 C	++++	++++	+++	+++
37 C	+	+	0	0
55 C	0	0	0	0

Table 21. Continued

Characteristics of Stock Cultures and Isolated Strains of Thiobacilli

	Oyster Pond Isolates			
	#MS-5	#MS-11	#MS-17	#MS-25
Growth at pH 3.0	0	+++	0	0
4.0	0	++++	0	0
5.0	0	++++	0	0
6.0	++	±	++	±
7.0	++++	0	++++	++++
8.0	+++	0	+++	+++
9.0	0	0	0	0
Growth on thiosulfate:	++++	++++	++++	++++
final pH:	3.9	2.1	4.7	6.5
Growth on sulfur:	++	++++	+	+
final pH:	6.0	1.1	6.4	6.9
Growth in organic media:				
Nutrient Broth:	0	0	0	0
Medium R :	0	0	0	0

Table 22.

Results of enrichment cultures for photosynthetic sulfur bacteria using Medium J for *Chlorobium* and Medium K for *Chromatium*. Two bottles of each medium inoculated with 1.0 ml water samples from various depths of the southern basin in 1968-69. Number of positive enrichments.

Depth, meters	Sampling Dates 9/8/68	5/20/69	7/15/69	8/14/69
		Chlorobium		
3.0			0	0
4.0			0	
4.5			0	0
5.0	2		2	2
5.4	2			
5.5	2		2	2
5.6	2			
6.0	2	0	2	2
6.2	2			
6.5		2	2	1
		Chromatium		
3.0			0	0
4.0			0	0
4.5			0	0
5.0	1		2	2
5.4	0			
5.5	2		2	0
5.6	1			
6.0	2	0	0	0
6.2	1			
6.5		0	0	0

Table 23.

Absorbance at 660 mμ and 770 mμ of methanol extracts of water samples from various depths of the southern basin in 1969. Sample (100 ml) filtered with glass fiber filter; filter extracted with 10 ml methanol; absorbance measured with 1 cm cuvettes.

	Absorbance at 660 mμ				
	Sampling Dates				
Depth, meters	7/2/69	7/13/69	7/27/69	8/14/69	8/24/69
1.5	0.188	0.214	0.282	0.354	0.246
4.5			0.082	0.188	0.166
5.0	0.068	0.188	0.240	0.238	0.162
5.5	0.146	0.378	0.866	0.510	0.256
6.0	0.126	0.160	0.160	0.192	0.202

	Absorbance at 770 mμ				
	Sampling Dates				
Depth, meters	7/2/69	7/13/69	7/27/69	8/14/69	8/24/69
1.5	0.060	0.038	0.042	0.038	0.026
4.5			0.050	0.038	0.028
5.0	0.034	0.066	0.036	0.028	0.028
5.5	0.028	0.042	0.034	0.042	0.040
6.0	0.024	0.042	0.032	0.034	0.050

Table 24.

Results of enrichment cultures for other chemolithotrophic bacteria. Medium L for methanol oxidizing bacteria; Medium M for hydrogen bacteria; Medium N for *Gallionella*; Medium O for *Ferrobacillus*; Medium P for ammonium-oxidizing nitrifying bacteria; Medium Q for nitrite oxidizing nitrifying bacteria. Three tubes of each medium inoculated with 1.0 ml water samples from various depths of the southern basin. Number of positive enrichments.

7/5/69

Depth, meters	Methanol Oxidizing Bacteria	Hydrogen Bacteria	Iron Bacteria: *Gallionella*	Iron Bacteria: *Ferrobacillus*	Nitrite Oxidizing Bacteria	Ammonium Oxidizing Bacteria
1.0	3	1	0	0	0	1
3.0	3	2	0	0	0	1
4.0	3	1	0	0	1	3
4.5	3	0	0	0	1	1
5.0	3	2	2	2	0	2
5.5	3	0	1	0	1	1
6.0	3	0	0	0	0	0
6.5	3	0	0	0	0	0

7/27/69

Depth, meters	Methanol Oxidizing Bacteria	Hydrogen Bacteria	Iron Bacteria: *Gallionella*	Iron Bacteria: *Ferrobacillus*	Nitrite Oxidizing Bacteria	Ammonium Oxidizing Bacteria
1.0	3	0	0	0	0	2
4.0	3	3	0	0	0	1
5.0	3	0	1	0	0	0
6.0	3	0	0	0	0	0

Table 25.

Enumeration of aerobic heterotrophic bacteria at various depths of the southern basin in 1968, using Nutrient Agar and Medium R. Plate Counts, 10^3/ml.

	Sampling Dates		
	7/29/68		9/22/68
Depth, meters	Nutrient Agar	Medium R	Nutrient Agar
1.5	5.0	2.6	3.6
3.0	4.6	2.2	
3.5	6.6	3.2	
4.0	7.1	4.1	6.9
4.5	3.0	2.4	
5.0	3.3	1.7	8.0
5.3			9.8
5.5			8.2
5.7			9.1
6.0			6.7
6.2			3.3

Table 26.

Enumeration of aerobic heterotrophic bacteria at various depths of the southern basin in 1969, using Nutrient Agar. Plate counts, 10^3/ml.

Depth, meters	Sampling Dates			
	3/27/69	4/23/69	5/20/69	6/24/69
0.0	7.2	71	14	10
1.0	8.4	87	15	15
2.0	6.6	50	21	17
3.0	7.4	38	15	12
4.0	7.9	14	16	8.2
5.0	4.1	20	19	6.4
5.0	3.1	13	23	8.3
6.5	1.9	18	12	5.5

Depth, meters	7/5/69	7/15/69	7/31/69	8/14/69
0.0	6.0	6.5	5.3	5.0
1.0	8.7	6.8	5.7	5.2
2.0	9.7	8.7	6.2	6.0
3.0	9.9	8.9	5.9	6.0
4.0	7.9	5.6	7.7	8.2
5.0	5.6	6.7	5.0	4.0
6.0	4.9	4.3	3.0	2.4
6.5	3.4	2.9	3.2	3.1

Table 27.

Enumeration of anaerobic heterotrophic bacteria at various depths of the southern basin in 1969, using Nutrient Agar plus 0.5 g/liter sodium thioglycollate. Plates incubated in 90% N_2, 10% CO_2; deep tubes overlaid with a 2 cm plug of sterile medium and incubated in the air. Plate counts or deep tube colony counts, 10^3/ml.

	Sampling Dates					
	3/27/69	5/20/69	7/5/69		7/15/69	8/14/69
Depth, meters	Plate Count	Plate Count	Plate Count	Deep Tube Count	Plate Count	Plate Count
0.0			0.12	0.01		
1.0			0.56	0.15	0.80	0.30
2.0			3.0	0.88		
3.0			3.0	1.2	1.8	1.3
4.0		2.0	4.0	1.7	3.6	4.0
5.0	2.1	2.1	4.2	4.3	4.2	4.9
6.0	3.1	1.7	5.2	4.9	4.5	1.9
6.5	2.1	2.5	1.2	1.8	1.5	1.6

Table 28.

Enumeration of dissimilatory sulfate-reducing bacteria at various depths of the southern basin water column and in the surface layers of the sediment in 1969 using Medium S. Deep tube colony counts/ml of water or mud.

Depth, meters	Sampling Dates 3/27/69	5/20/69	7/5/69	7/15/69
4.0			0	0
4.5			0	1.0×10^1
5.0			4.3×10^1	9.0×10^1
5.5		0	3.0×10^2	2.5×10^2
6.0	0	2.0×10^1	4.0×10^2	3.8×10^2
6.5	1.3×10^2	4.0×10^2	5.2×10^2	3.9×10^2
Sediment Surface	1.1×10^4	1.8×10^4	5.7×10^4	5.9×10^4

Table 29.

Enumeration of non-specific sulfate-reducing bacteria at various depths of the southern basin water column and in the surface layers of the sediment on 5/20/69 and 7/15/69. Medium T for aerobic non-specific sulfate-reducing bacteria; Medium U for anaerobic non-specific sulfate-reducing bacteria. Deep tube colony counts/ml of water or g of mud.

	Aerobic (Medium T)		Anaerobic (Medium U)	
	Sampling Dates		Sampling Dates	
Depth, meters	5/20/69	7/15/69	5/20/69	7/15/69
1.5	1.0×10^1	0.7×10^1	0	0
4.5	4.0×10^1	3.0×10^1	1.0×10^1	0.7×10^1
5.5	3.0×10^1	4.0×10^1	0	1.8×10^2
6.5	4.0×10^1	1.0×10^1	1.1×10^2	4.6×10^2
Sediment Surface	6.2×10^2	5.8×10^2	4.3×10^3	4.7×10^3

Table 30.

Growth of colonies from media designed for dissimilatory sulfate-reducing bacteria and non-specific sulfate-reducing bacteria. Ten colonies were picked from tubes of each medium (S, T, and U) and stabbed into all three of the media. Tubes were inspected daily for growth and sulfide production.

Growth and sulfide Production in:	Medium S		Medium T		Medium U	
	Number of Colonies		Number of Colonies		Number of Colonies	
Colonies picked from:	Growing	Producing H_2S	Growing	Producing H_2S	Growing	Producing H_2S
Medium S	6	6	0	0	1	1
Medium T	1	0	10	10	4	2
Medium U	3	1	6	4	9	8

Table 31.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 7/29/68, with and without the addition of 0.4 g/liter $Na_2S_2O_3 \cdot 5H_2O$.

			Without Added Thiosulfate			With Added Thiosulfate		
Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -blank	L-D cpm	mg C/m^3/hr	cpm -blank	L-D cpm	mg C/m^3/hr
1.5	L	7,770	6,748	6,672	55.4	7,333	7,202	59.8
"	D	"	76		0.63	131		1.09
3.0	L	8,730	2,030	1,986	18.6	2,095	1,970	18.5
"	D	"	44		0.41	125		1.17
3.5	L	10,130	1,888	1,760	19.1	1,754	1,636	17.7
"	D	"	128		1.39	118		1.28
4.0	L	11,790	1,419	1,197	15.1	1,415	1,145	14.5
"	D	"	222		2.80	270		3.41
4.5	L	14,100	897	561	8.47	1,120	201	3.04
"	D	"	336		5.08	919		13.9
5.0	L	38,200	702	61	2.49	951	257	10.5
"	D	"	641		26.2	694		28.4

N = 4.5 hr; F = 20; R = 4.36 x 10^6 cpm; Background = 62 cpm

Table 32.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 9/21/68.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg $C/m^3/hr$
1.5	L	4,870	1,680	1,737	1,702	21.6
"	L		1,893			
"	D	"	56	35		1.45
"	D		13			
4.0	L		4,558	4,416	4,422	108
"	L	9,360	4,273			
"	D	"	2	-6		0
"	D		-14			
5.0	L	9,800	3,912	3,795	3,764	105
"	L		3,677			
"	D	"	37	31		0.82
"	D		24			
5.3	L	10,020	2,176	2,313	2,279	59.7
"	L		2,449			
"	D	"	26	44		1.15
"	D		61			

Table 32. Continued

Productivity studies, southern basin, 9/21/68.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
5.5	L	10,190	1,584	1,388	1,348	35.7
"	L		1,191			
"	D	"	25	40		1.06
"	D		54			
5.7	L	10,620	1,193	1,044	927	25.7
"	L		894			
"	D	"	113	117		3.25
"	D		121			
6.0	L	23,560	925	856	154	9.58
"	L		787			
"	D	"	679	702		43.0
"	D		725			
6.2	L	71,300	546	516	124	23.0
"	L		486			
"	D	"	458	392		73.8
"	D		325			

N = 3.5 hr; F = 20; R = 2.30 x 10^6 cpm; Background = 41 cpm

Table 33.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 4/22/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	7,000	2,214	2,378	2,249	36.0
"	L	"	2,542			
"	D		129			2.06
5.5	L	6,600	176		97	1.46
"	D	"	108			
"	D		50	79		1.19
6.0	L	7,700	176		66	1.16
"	D	"	115			
"	D		105	110		1.93

N = 4.0 hr; F = 20; R = 2.30 x 10^6 cpm; Background = 33 cpm

Table 34

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 5/22/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	8,100	6,692	6,982	6,766	73.3
"	L	"	7,271			
"	D		216			2.34
5.5	L	8,600	2,609		2,532	29.1
"	D	"	74	77		0.89
"	D		80			
6.0	L	7,800	728		652	6.80
"	D	"	71	76		0.79
"	D		80			

N = 4.0 hr; F = 20; R = 3.92 x 10^6 cpm; Background = 36 cpm

Table 35.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 6/25/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	8,100	3,164	3,180	3,143	70.0
"	L		3,196			
"	D	"	23	37		0.82
"	D		50			
5.0	L	9,700	1,365	1,222	1,200	32.0
"	L		1,079			
"	D	"	19	22		0.59
"	D		24			
5.5	L	11,800	511	568	551	17.9
"	L		602			
"	D	"	24	17		0.55
"	D		9			
6.0	L	32,500	548	469	445	39.7
"	L		389			
"	D	"	20	24		2.14
"	D		27			

N = 3.5 hr; F = 20; R = 2.18 x 10^6 cpm; Background = 39 cpm

Table 36

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 7/5/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg $C/m^3/hr$
1.5	L	8,200	2,738	2,656	2,649	52.2
"	L		2,573			
"	D	"	17	7		0.13
"	D		-3			
4.0	L	8,700	1,078	1,113	1,079	22.5
"	L		1,149			
"	D	"	43	34		0.71
"	D		24			
4.5	L	12,100	604	671	667	19.4
"	L		738			
"	D	"	13	4		0.12
"	D		-5			
5.0	L	22,700	245	224	196	10.7
"	L		203			
"	D	"	31	28		1.53
"	D		25			

N = 4.0 hr; F = 20; R = 2.18×10^6 cpm; Background = 40 cpm

Table 37.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 7/19/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	8,300	2,925	2,770	2,766	55.0
"	L		2,615			
"	D	"	14	4		0.08
"	D		-7			
4.5	L	16,300	448	474	443	17.4
"	L		500			
"	D	"	18	31		1.21
"	D		43			
5.0	L	22,700	535	478	200	10.9
"	L		420			
"	D	"	332	278		15.2
"	D		223			
5.5	L	34,200	314	295	59	4.85
"	L		276			
"	D	"	222	236		19.4
"	D		250			
6.0	L	48,200	43	29	10	1.16
"	L		15			
"	D	"	26	19		2.20
"	D		12			

N = 4.0 hr; F = 20; R = 2.18×10^6 cpm; Background = 41 cpm

Table 38.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 7/28/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	8,600	1,211	2,219	2,239	46.2
"	L		2,316			
"	D	"	7	-20		0
"	D		-47			
4.5	L	17,200	425	479	368	15.2
"	L		532			
"	D	"	70	111		4.58
"	D		152			
5.0	L	22,300	345	326	-16	0
"	L		302			
"	D	"	334	342		18.3
"	D		350			
5.5	L	37,400	300	281	130	11.7
"	L		261			
"	D	"	136	151		13.6
"	D		165			

N = 4.0 hr; F = 20; R = 2.18 x 10^6 cpm; Background = 38 cpm

Table 39.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the southern basin on 8/24/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg $C/m^3/hr$
1.5	L	8,100	2,434	2,550	2,492	43.2
"	L		2,665			
"	D	"	70	58		1.01
"	D		46			
3.0	L	9,100	961	889	878	17.1
"	L		816			
"	D	"	15	11		0.21
"	D		6			
4.0	L	12,200	667	574	427	11.1
"	L		480			
"	D	"	134	147		3.85
"	D		160			
5.0	L	20,100	635	613	175	7.50
"	L		591			
"	D	"	426	438		18.9
"	D		449			
6.0	L	43,500	94	63	32	2.99
"	L		31			
"	D	"	13	31		2.89
"	D		49			

N = 4.5 hr; F = 20; R = 2.18×10^6 cpm; Background = 38 cpm

Table 40.

Productivity studies in the presence of varying concentrations of a single substrate with water collected from 5 m in the southern basin on 8/16/69. Populations centrifuged at 5000 G for 10 min and resuspended in Basal Medium to which the substrates were added. Incubation in the dark at 20 C. Fixation in cpm/liter (minus blank).

Substrate	Amounts Added					
No added Substrates	720 1,400 1,120 1,240	1,120				
	Bubble 30 sec		60 sec		120 sec	
H_2 "	1,320 1,000	1,160	1,560 1,320	1,440	1,400 1,200	1,300
	1 m M		5 m M		10 m M	
Fe^{++} "	720 400	560	---		---	
NH_4^+ "	1,120 1,120	1,120	800 1,080	940	1,600 1,200	1,400
NO_2^- "	800 1,120	960	440 1,040	740	600 880	740
$S^=$ "	1,000 1,240	1,120	1,800 1,560	1,680	1,280 1,640	1,460

Table 40. Continued

Productivity studies in the presence of varying concentrations of a single substrate - southern basin, 5.0 m - 8/16/69.

	1 m M		5 m M		10 m M	
S° (+100 ppm Tween 80)	1,360	1,440	1,960	1,840	1,360	1,520
"	1,520		1,720		1,680	
$S_2O_3^{=}$	1,080	1,160	2,080	2,220	2,680	2,880
"	1,240		2,360		3,080	
CH_3OH	1,600	1,680	4,920	4,400	7,840	7,180
"	1,760		3,880		6,520	
Glucose	2,360	2,240	3,400	3,820	6,240	5,960
"	2,120		4,240		5,680	
All of above	2,880	2,640	---		---	
	2,400					

N = 3.0 hr; 1.0 μc $NaHC^{14}O_3$/50 ml; Background = 38 cpm

Table 41.

Productivity studies with added _T. thioparus_ cells in water samples from 4.5 and 5.0 m in the southern basin in 7/28/69. 0, 10^2, 10^3, and 5 x 10^3 cells/100 ml of a pure culture were added to samples. Incubation in the dark.

Depth, meters	Total Inorganic Carbon, mg C/m^3	Added _T. thioparus_ cells/100 ml	cpm -Blank	Mean cpm	mg $C/m^3/hr$
4.5	17,200	0	70	111	4.58
"		"	152		
"	"	10^2	100	113	4.67
"		"	126		
"	"	5 x 10^2	104	136	5.61
"		"	167		
"	"	10^3	115	142	5.86
"		"	69		
"	"	5 x 10^3	89	115	4.74
"		"	140		

Table 41. Continued

Productivity studies with added *T. thioparus* cells - southern basin, 4.5, 5.0 m - 7/28/69

Depth, meters	Total Inorganic Carbon, mg C/m^3	Added *T. thioparus* cells/100 ml	cpm -Blank	Mean cpm	mg C/m^3/hr
5.0	22,300	0	334	342	18.3
"		"	350		
"	"	10^2	372	363	19.5
"		"	354		
"	"	5×10^2	335	324	17.4
"		"	312		
"	"	10^3	380	342	18.3
"		"	297		
"	"	5×10^3	346	362	19.4
"		"	377		

N = 4.0 hr; F = 20; R - 2.18×10^6 cpm; Background = 38 cpm

Table 42.

Productivity studies with added *T. thioparus* cells in water samples from 5.5 and 6.2 m in the southern basin on 11/8/69. 0, 10^2, 10^3, 10^4, 10^5, and 10^6 cells/100 ml of a pure culture were added to samples. Incubation in the dark at 20 C.

Depth, meters	Total Inorganic Carbon, mg C/m^3	Added *T. thioparus* cells/100 ml	cpm -Blank	Mean cpm	mg C/m^3/hr
5.5	19,100	0	54	48	2.53
"		"	42		
"	"	10^2	57	56	2.94
"		"	55		
"	"	10^3	65	65	3.42
"	"	10^4	51	54	2.84
"		"	57		
"	"	10^5	67	66	3.47
"		"	64		
"	"	10^6	62	61	3.21
"		"	60		
6.2	34,100	0	62	66	6.20
"		"	70		
"	"	10^2	53	40	3.76
"		"	26		
"	"	10^3	48	35	3.29
"		"	22		
"	"	10^4	55	52	4.88
"		"	48		
"	"	10^5	42	32	3.01
"		"	22		
"	"	10^6	49	53	4.98
"		"	56		

N = 3.5 hr; F = 20; R = 2.18 x 10^6 cpm; Background = 50 cpm

Table 43.

Productivity studies with a pure culture of *T. thioparus* in Basal Medium plus 0.4 g/liter $Na_2S_2O_3 \cdot 5H_2O$ and Medium B on 3/5/70. 0, 10^2, 10^4, 10^6, and 10^8 cells/100 ml of a pure culture were added to sterile media. Incubation in the dark at 20 C.

Medium	Total Inorganic Carbon, mg C/m^3	Added *T. thioparus* cells/100 ml	cpm -Blank	Mean cpm	mg $C/m^3/hr$
Basal + 0.4 g/liter $Na_2S_2O_3 \cdot 5H_2O$	10,800	0	0.0	0.5	0
		"	1.0		
"	"	10^2	0.4	0	0
		"	-0.4		
"	"	10^4	-4.7	-3.0	0
		"	-1.2		
"	"	10^6	3.5	0.3	0
		"	-3.0		
"	"	10^8	115	126	2.34
		"	137		
B	1,230	0	-1.5	-0.2	0
		"	1.2		
"	"	10^2	-0.6	-1.6	0
		"	-2.6		
"	"	10^4	0.5	1.2	0
		"	1.8		
"	"	10^6	8.0	8.5	0.02
		"	9.0		
"	"	10^8	1,401	1,423	3.02
		"	1,444		

N = 5.0 hr; F = 5; R = 6.10×10^5 cpm; Background = 39 cpm

Table 44.

Oxidation of S^{35}-sulfide in 24 hr in water samples collected from 1.5 m in the southern basin on 7/5/69. Incubation in the light and dark, presence and absence of formalin.

	Light		Dark		Light & Formalin		Dark & Formalin	
	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total
Sulfide	218 11	2.5	142 102	2.6	77 199	2.9	122 42	1.7
"Cells"	307 234	5.7	423 341	8.0	376 304	7.1	299 282	6.1
Sulfur	658 785	15.1	525 655	12.4	727 805	16.0	783 721	15.7
"Soluble" (Assuming Total = 4,780 cpm)	-107 105	0.0	93 -90	0.0	61 -19	0.6	38 86	1.3
Sulfate	3,704 3,645	76.7	3,598 3,772	77.0	3,539 3,491	73.4	3,538 3,649	75.2

Background = 42 cpm; 0.0 ppm sulfide at 1.5 m; 0.52 ppm sulfide at 5.0 m, 9.3 ppm sulfide at 6.0 m

Initial Sulfide:

	Sulfide		"Cells"		Sulfur		Sulfate		Total	
cpm	4421 4728	4575	75 59	67	70 44	57	93 69	81	4659 4900	4780
%	95.7		1.4		1.2		1.7		100	

Table 44. Continued

Oxidation of S^{35}-sulfide
7/5/69 - 5.0 m

	Light		Dark		Light & Formalin		Dark & Formalin	
	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total
Sulfide	401 355	7.9	512 671	12.3	796 885	17.6	1,013 881	19.8
"Cells"	339 403	7.8	566 583	12.0	603 657	13.2	757 877	17.1
Sulfur	839 742	16.7	945 1,082	21.3	874 935	18.9	1,096 992	21.9
"Soluble" (Assuming Total = 4,780 cpm)	68 177	2.6	169 251	4.4	277 312	6.2	283 262	5.9
Sulfate	3,133 3,103	65.0	2,588 2,193	50.1	2,330 1,991	43.1	1,632 1,768	35.3

Table 44. Continued

Oxidation of S^{35}-sulfide
7/5/69 - 6.0 m

	Light		Dark		Light & Formalin		Dark & Formalin	
	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total
Sulfide	327 288	6.5	377 392	8.1	499 ---	10.4	463 467	9.7
"Cells"	171 228	4.2	288 329	6.5	195 ---	4.1	182 233	4.3
Sulfur	78 103	1.9	53 41	1.0	76 ---	1.6	79 61	1.5
"Soluble" (Assuming Total = 4,780 cpm)	3,224 3,273	68.1	3,169 3,282	67.5	3,257 ---	68.2	3,303 3,200	68.1
Sulfate	980 888	19.3	893 736	16.9	753 ---	15.7	803 819	16.4

Table 45.

Oxidation of S^{35}-sulfide in 24 hr in water samples collected from 5.0 m in the southern basin on 8/15/69. Incubation in the light and dark, presence and absence of formalin.

	Light		Dark		Light & Formalin		Dark & Formalin	
	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total	cpm	Mean % of Total
Sulfide	427 477	13.6	562 504	16.1	698 660	20.4	671 649	19.8
"Cells"	62 78	2.1	88 65	2.3	46 57	1.6	59 38	1.5
Sulfur	185 204	5.9	267 314	8.8	394 334	11.0	375 432	12.2
"Soluble" (Assuming Total = 3,320 cpm)	465 526	14.9	621 681	19.6	687 729	21.3	759 670	21.5
Sulfate	2,181 2,035	63.5	1,782 1,756	53.2	1,495 1,540	45.7	1,456 1,532	45.0

Background = 47 cpm; 4.7 ppm sulfide at 5.0

Initial Sulfide:

	Sulfide		"Cells"		Sulfur		Sulfate		Total	
cpm	2890 3364	3127	2 14	8	115 144	130	31 79	55	3038 3601	3320
%		94.3		0.2		3.9		1.6		100

Table 46.

Oxidation of S^{35}-sulfide in 24 hr with added *T. thioparus* cells in water samples collected from 6.2 m in the southern basin on 11/8/69. 0, 10^2, 10^4, and 10^6 cells/100 ml of a pure culture were added to the samples. Incubation in the dark at 20 C in the presence and absence of formalin.

	0 cells/100 ml		0 cells/100 ml + Formalin		10^2 cells/100 ml		10^2 cells/100 ml + Formalin	
	cpm	% of Total	cpm	% of Total	cpm	% of Total	cpm	% of Total
Sulfide	632 697	38.7	617 629	36.3	617 719	38.9	652 613	36.8
"Cells"	2 22	0.7	13 29	1.2	37 14	1.5	-4 29	0.8
Sulfur	159 199	10.4	187 290	13.9	309 162	13.7	280 143	12.3
"Soluble" (Assuming Total = 1,718 cpm)	149 135	8.3	175 149	9.4	109 162	7.9	180 139	9.6
Sulfate	743 665	41.9	726 608	39.2	646 661	38.0	610 794	40.5

Background = 50 cpm; 2.4 ppm sulfide at 6.2 m

Initial Sulfide:

	Sulfide		"Cells"		Sulfur		Sulfate		Total	
cpm	1733 1433	1583	20 -6	7	75 62	69	21 99	60	1849 1588	1718
%	92.1		0.4		4.0		3.5		100	

Table 46. Continued

Oxidation of S^{35}-sulfide with added <u>T. thioparus</u>
11/8/69 - 6.2 m

	10^4 cells/100 ml		10^4 cells/100 ml + Formalin		10^6 cells/100 ml		10^6 cells/100 ml + Formalin	
	cpm	% of Total	cpm	% of Total	cpm	% of Total	cpm	% of Total
Sulfide	581 700	37.3	702 627	38.7	617 701	38.3	601 690	37.6
"Cells"	32 10	1.2	22 14	1.1	-6 20	0.4	18 0	0.5
Sulfur	179 209	11.3	237 201	12.7	210 261	13.7	197 209	11.8
"Soluble" (Assuming Total = 1,718 cpm)	130 138	7.8	152 147	8.7	120 108	6.6	140 162	8.8
Sulfate	796 661	42.4	605 629	38.8	775 628	41.0	762 657	41.3

Table 47.

Oxidation of S^{35}-sulfide in 24 hr with a pure culture of T. thioparus in Basal Medium plus 1.0 ppm sulfide on 3/11/70. 0, 10^2, 10^4, 10^6, and 10^8 cells/100 ml of a pure culture were added to sterile medium. Incubation in the dark at 20 C in the presence and absence of formalin.

	0 cells/100 ml		0 cells/100 ml + Formalin		10^2 cells/100 ml		10^2 cells/100 ml + Formalin	
	cpm	% of Total	cpm	% of Total	cpm	% of Total	cpm	% of Total
Sulfide	97 89	17.7	105 90	18.9	95 94	18.1	91 110	19.2
"Cells"	0 15	1.4	8 2	1.0	12 6	1.7	9 7	1.5
Sulfur	111 129	22.9	115 119	22.5	109 113	21.1	120 122	23.1
Soluble	52 64	11.1	48 57	10.2	59 39	9.3	58 44	9.7
Sulfate	268 222	46.9	256 238	47.4	255 268	49.8	253 236	46.5

Background = 22 cpm

Initial Sulfide:

	Sulfide		"Cells"		Sulfur		Sulfate		Soluble		Total	
cpm	479 509	494	0 9	4.5	19 6	12.5	37 29	33	-8 13	2.5	527 566	547
%	90.4		0.8		2.3		6.0		0.5		100	

Table 47. Continued

Oxidation of S^{35}-sulfide with pure culture *T. thioparus*
3/11/70

	10^4 cells/100 ml		10^4 cells/100 ml + Formalin		10^6 cells/100 ml		10^6 cells/100 ml + Formalin	
	cpm	% of Total	cpm	% of Total	cpm	% of Total	cpm	% of Total
Sulfide	98 96	18.4	89 97	17.9	94 104	19.2	100 93	18.3
"Cells"	-1 14	1.2	12 12	2.3	12 3	1.5	5 8	1.2
Sulfur	116 109	21.5	124 114	22.9	120 112	22.5	118 117	22.4
Soluble	59 54	10.8	41 52	9.0	47 59	10.3	56 50	10.0
Sulfate	257 251	48.1	243 253	47.9	241 237	46.5	253 256	48.1

Table 47. Continued

Oxidation of S^{35}-sulfide with pure culture _T. thioparus_
3/11/70

	10^8 cells/100 ml		10^8 cells/100 ml + Formalin	
	cpm	% of Total	cpm	% of Total
Sulfide	78 84	15.2	90 92	17.6
"Cells"	12 4	1.5	6 2	0.8
Sulfur	102 111	20.1	126 115	23.3
Soluble	7 12	1.8	62 48	10.6
Sulfate	341 313	61.4	242 252	47.7

Table 48.

Analyses of sediments collected at station 18 in the southern basin on 8/15/69, 11/13/69, and 1/15/70.

	Sampling Dates		
	8/15/69	11/13/69	1/15/70
Water, % Wet Weight	76.8	77.0	74.8
Organic Matter, % Dry Weight	13.3	15.0	17.2
Chloride, ppt Dry Weight	14.9	14.5	12.0
Water-Soluble Sulfide, ppm Dry Weight	246	110	109
Acid-Soluble Sulfide, ppm Dry Weight	439	183	209
Pyrite-S, ppm Dry Weight	1,680		1,760
Elemental-S, ppm Dry Weight	10.2		8.7
Sulfate-S, ppm Dry Weight	453	527	432
Total Inorganic-S, ppm Dry Weight	2,828		2,519
Predicted Sulfate-S, ppm Dry Weight	693	673	557
Excess S, ppm Dry Weight	2,135		1,962
Ratio, $SO_4^{=}/Cl^-$	0.090	0.109	0.108
Ratio, Total Inorganic S (as $SO_4^{=}$)/Cl^-	0.569		0.629

Ratio, $SO_4^{=}/Cl^-$ of seawater 0.1394

Table 49.

Secchi disc readings (D) in meters, computed extinction coefficients (k), and 1% light level in meters, for the northern basin in 1968-79.

Date	D, Meters	k	1% Light Level, Meters
7/19/68	1.7	1.000	4.6
6/ 2/69	1.1	1.544	3.0
7/ 2/69	1.8	0.944	4.9
7/27/69	1.5	1.133	4.1
8/24/69	0.9	1.890	2.4
Mean	1.4	1.213	3.8

Table 50.

Temperature readings, C, at various depths of the northern basin in 1968-69.

Depth, meters	Sampling Dates 7/19/68	6/2/69	7/2/69	7/27/69	8/24/69
0.0	27.0	21.5	22.5	21.3	26.9
0.5		20.8	22.3	21.3	26.0
1.0	27.0	20.3	22.3	21.4	24.4
1.5		20.0	22.2	21.2	23.8
2.0	24.2	19.9	22.1	21.2	23.3
2.5		19.7	21.9	21.1	23.1
3.0	21.6	18.0	21.6	20.9	22.7
3.5		17.3	20.0	19.8	22.1
3.6	19.8				
3.7	19.1				
3.8	18.9				
4.0		17.0	19.0	17.9	20.7
4.1	18.1				
4.2		16.9	18.6	17.8	19.4

Table 51.

Chlorinity determinations, ppt, at various depths of the northern basin in 1968-69.

	Sampling Dates				
Depth, meters	7/19/68	6/2/69	7/2/69	7/27/69	8/24/69
0.0	0.72	0.64	0.71	0.76	0.82
0.5		0.63	0.72	0.74	0.80
1.0	0.71	0.95	0.74	0.73	0.78
1.5		0.70	0.68	0.74	0.79
2.0	0.68	0.67	0.72	0.76	0.77
2.5		0.92	0.72	0.72	0.73
3.0	0.57	0.68	0.69	0.70	0.73
3.5		0.78	0.62	0.56	0.69
4.0		0.68	0.57	0.86	0.69
4.1	0.61				
4.2		0.73	0.68	0.67	0.67

Table 52.

pH measurements at various depths of the northern basin in 1969.

Depth, meters	Sampling Dates 6/2/69	7/27/69	8/24/69
0.0	7.80	7.69	9.58
0.5	7.86	7.83	9.56
1.0	7.80	9.02	9.63
1.5	7.50	7.98	9.47
2.0	7.48	7.46	8.92
2.5	7.14	7.23	7.97
3.0	6.94	7.07	7.22
3.5	6.72	6.94	6.68
4.0	6.61	6.73	6.42
4.2	6.70	6.80	6.44

Table 53.

Dissolved oxygen determinations, ppm, at various depths of the northern basin in 1969.

Depth, meters	Sampling Dates 6/2/69	7/2/69	7/27/69	8/24/69
0.0	9.50	8.97	9.58	9.67
1.0	9.70	9.46	10.38	9.96
2.0	8.52	8.77	9.71	8.42
3.0	5.92	5.98	6.10	5.86
3.5			3.37	4.43
4.0	2.46	1.97	0.00	0.00
4.2	1.95	0.00		

Table 54.

Sulfide determinations, ppm sulfide-S, at various depths of the northern basin in 1969.

Depth, meters	Sampling Dates 6/2/69	7/2/69	7/27/69	8/24/69
3.5			00.0	00.0
4.0	00.0	00.0	3.3	5.0
4.2	00.0	00.0	10.2	17.2

Table 55.

Thiosulfate determinations, ppm thiosulfate-S, at various depths of the northern basin in 1969.

Depth, meters	Sampling Dates 6/2/69	7/2/69	7/27/69	8/24/69
3.5	0	0	0	0
4.0	0	0	0.35	0.54
4.2	0	0	0.55	0.60

Table 56.

Polythionate determinations, ppm tetrathionate-S, at various depths of the northern basin 1969.

Depth, meters	Sampling Dates 6/2/69	7/2/69	7/27/69	8/24/69
3.5	0	0	0	0
4.0	0	0	0	0
4.2	0	0	0.50	0.45

Table 57.

Sulfite determinations, ppm sulfite-S, at various depths of the northern basin in 1969.

Depth, meters	Sampling Dates		
	6/2/69	7/2/69	7/27/69
3.5			0.0
4.0	0.0	0.0	0.09
4.2	0.0	0.11	0.15

Table 58.

Enumeration of *T. thioparus* at various depths of the northern basin in 1969. MPN/100 ml using Medium B.

Depth, meters	Sampling Dates 7/5/69	7/31/69	8/25/69
1.5		20	
3.0	0		
3.5		20	50
4.0	0	120	
4.2	130	170	220

Table 59.

Enumeration of *T. thiooxidans* at various depths of the northern basin in 1969. MPN/100 ml using Medium E.

Depth, meters	Sampling Dates 7/5/69	7/31/69	8/25/69
1.5		0	
3.0	0		
3.5		0	0
4.0	0	50	
4.2	0	0	50

Table 60.

Enumeration of *T. denitrificans* at various depths of the northern basin in 1969. MPN/100 ml using Medium H.

Depth, meters	Sampling Dates 7/5/69	7/31/69	8/25/69
1.5		0	
3.0	0		
3.5		0	0
4.0	0	0	
4.2	0	0	20

Table 61.

Enumeration of heterotrophic bacteria at various depths of the northern basin on 7/5/69 and 7/31/69. Aerobic heterotrophic bacteria using Nutrient Agar. Anaerobic heterotrophic bacteria using Nutrient Agar plus 0.5 g/liter sodium thioglycollate. Plate counts, thousands/ml.

Depth, meters	Aerobic 7/5/69	Aerobic 7/31/69	Anaerobic 7/5/69
0.0	9.2	8.0	
1.0	9.2	8.9	0.5
2.0	8.1	7.3	
3.0	6.6	7.6	2.1
4.0	3.2	1.8	1.2
4.2	1.2	0.9	0.7

Table 62.

Enumeration of dissimilatory sulfate-reducing bacteria at various depths of the northern basin water column and in the surface layers of the sediment on 7/5/69, using Medium S. Deep tube colony counts/ml of water or g of mud.

Depth, meters	Sampling Date 7/5/69
3.5	1.0 c 10^1
4.0	1.2 x 10^2
4.2	4.0 x 10^2
Sediment Surface	9.8 x 10^3

Table 63.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the northern basin on 7/5/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	7,900	1,702	1,790	1,766	33.6
"	L		1,878			
"	D	"	30	24		0.46
"	D		17			
3.5	L	8,600	629	579	553	11.4
"	L		528			
"	D	"	37	26		0.54
"	D		14			
4.0	L	11,900	232	277	257	7.35
"	L		321			
"	D	"	8	20		0.57
"	D		32			

N = 4.0 hr; F = 20; R = 2.18 x 10^6 cpm; Background = 40 cpm

Table 64.

Productivity studies using $NaHC^{14}O_3$ with water samples from various depths of the northern basin on 7/28/69.

Depth, meters	Light or Dark Bottle	Total Inorganic Carbon, mg C/m^3	cpm -Blank	Mean cpm	L-D cpm	mg C/m^3/hr
1.5	L	8,200	2,160	2,035	2,038	40.2
"	L		1,910			
"	D	"	-10	-3		0
"	D		5			
3.5	L	18,200	224	208	185	8.11
"	L		191			
"	D	"	36	23		1.01
"	D		9			
4.0	L	24,900	442	473	139	8.34
"	L		503			
"	D	"	360	334		20.0
"	D		307			

N = 4.0 hr; F = 20; R = 2.18 x 10^6 cpm; Background = 38 cpm

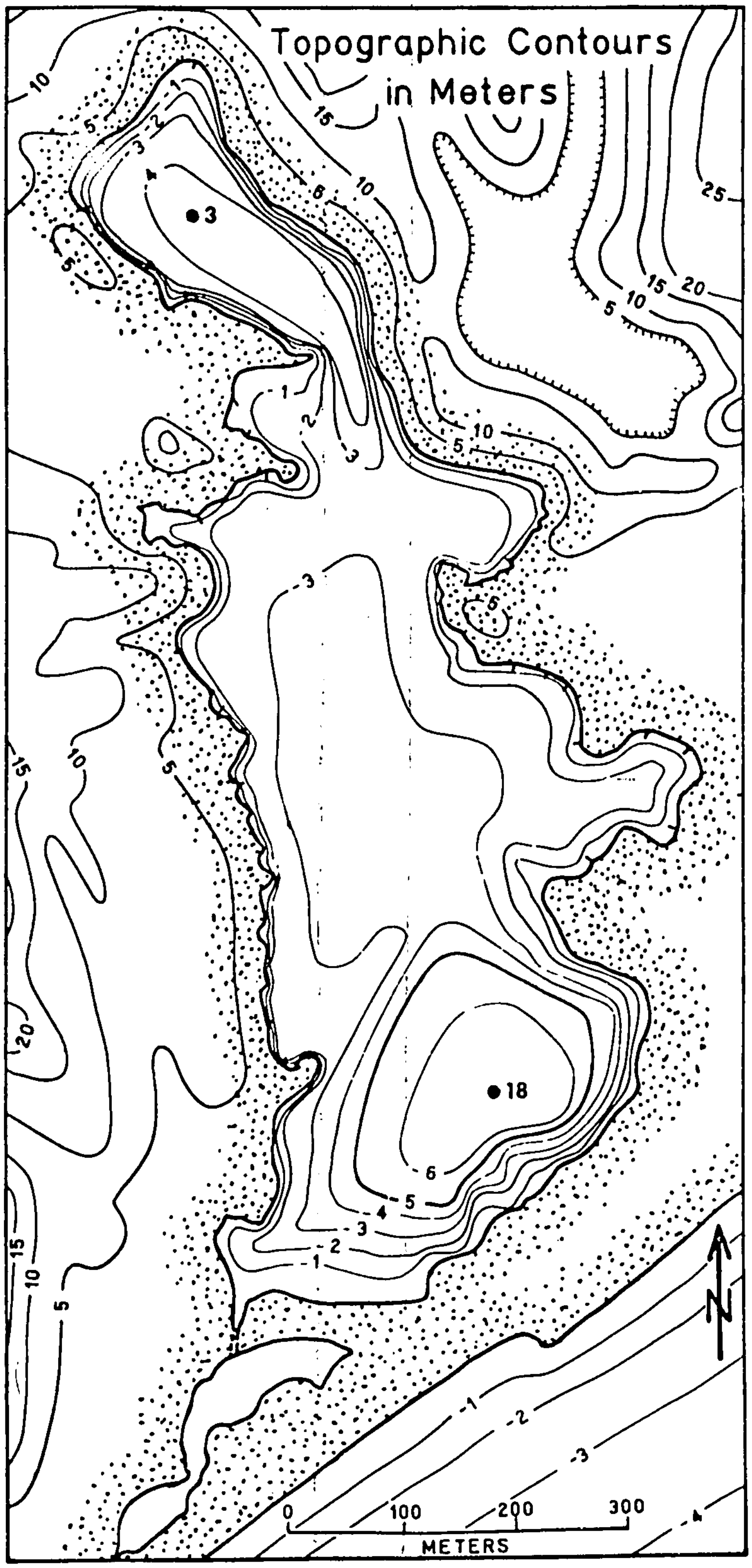


Fig. 1. Bathymetry and topography of Oyster Pond and vicinity (courtesy of K. O. Emery).

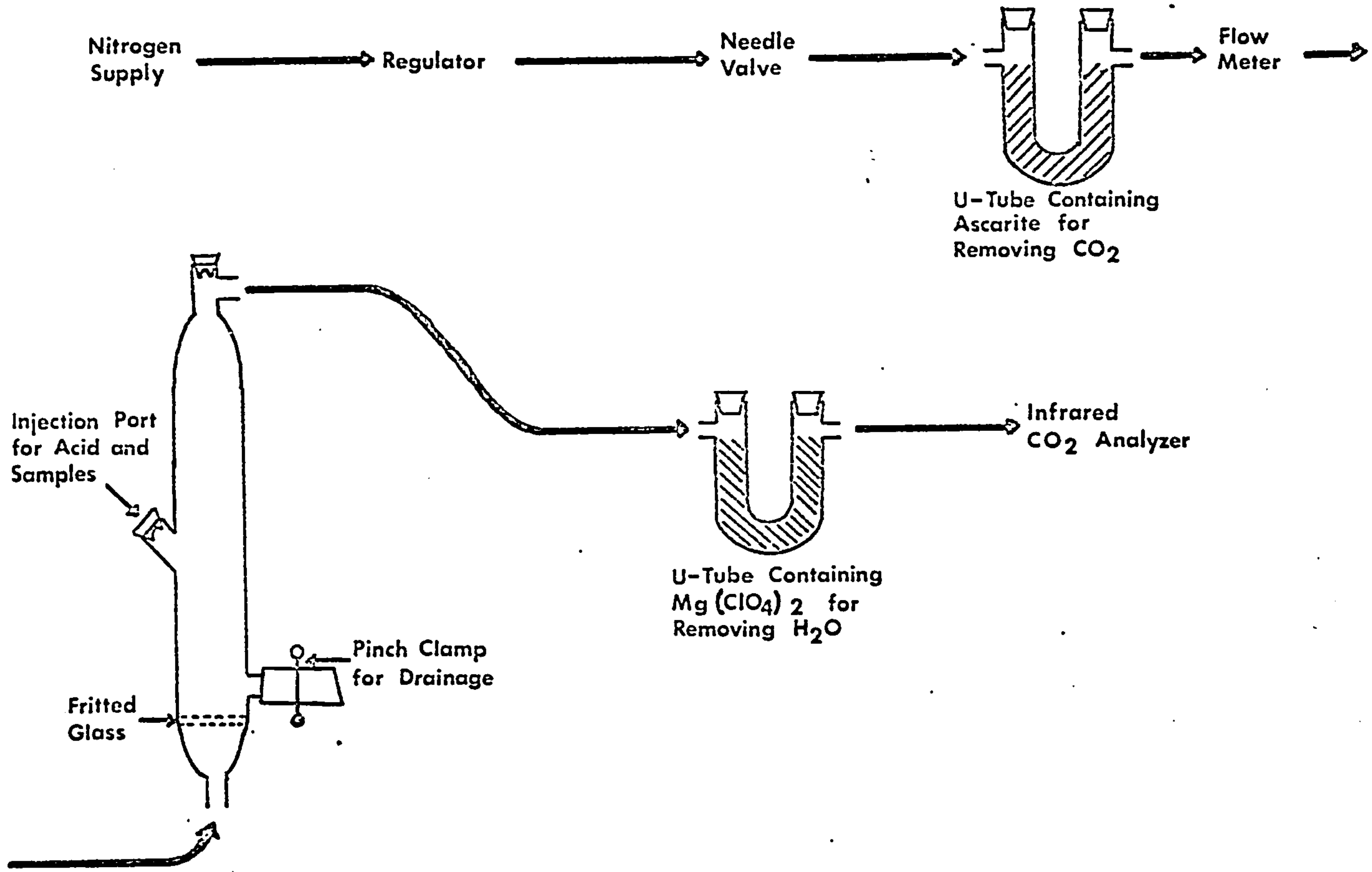


Fig. 2. Schematic diagram of apparatus used in the analysis of total inorganic carbon in water samples.

Filter aliquots with
0.45 μ Millipore membranes

(Count #1 = $S^{=}$, S°, Cells)

Precipitate $SO_4^{=}$ in
filtrate with $BaCl_2$

Extract $S^{=}$
with HCl fumes

(Count #2 = S°, Cells)

Filter with 0.45 μ
Millipore membranes
(Count #4 = $SO_4^{=}$)

Extract S°
with benzene

(Count #3 = Cells)

Fig. 3. Flow Chart for S^{35} Assay Procedure

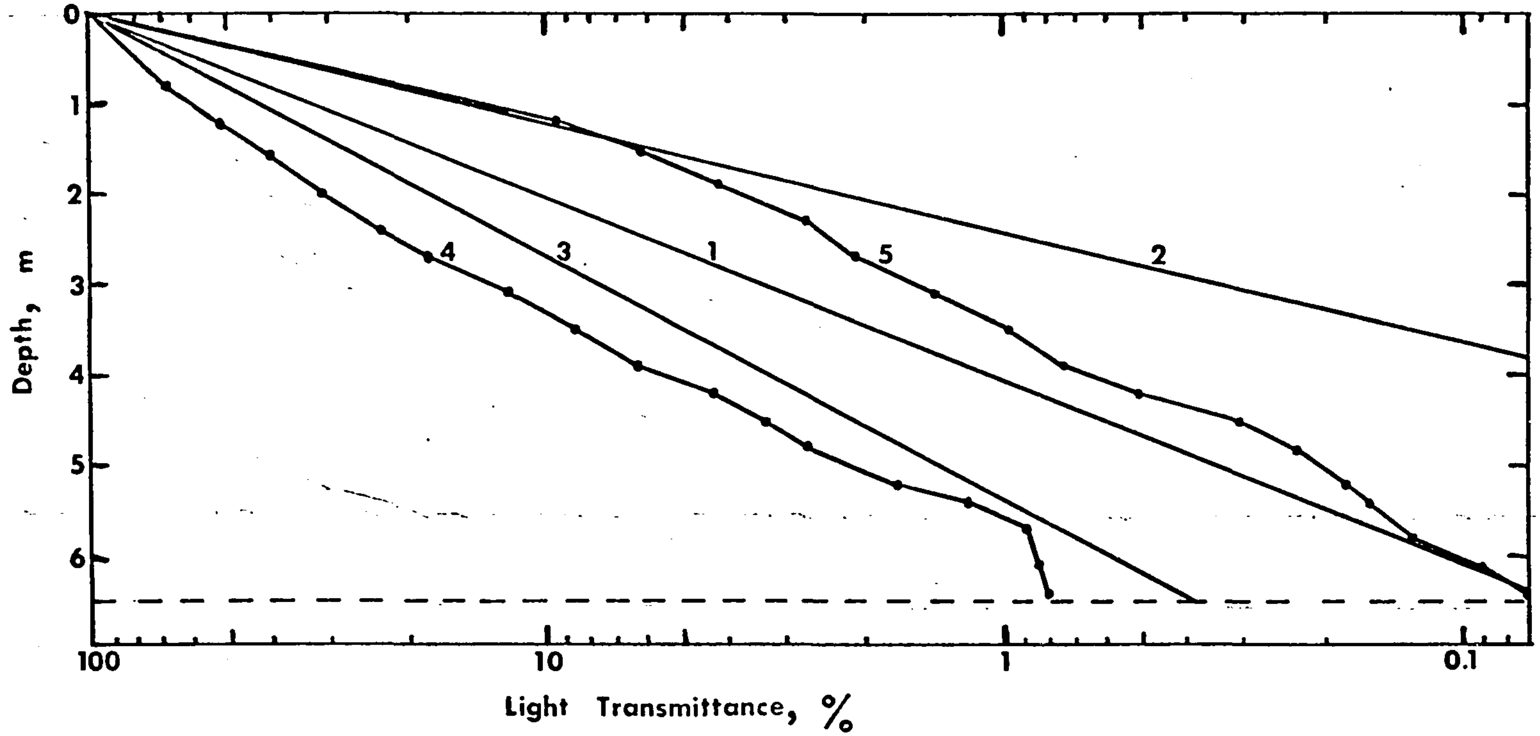


Fig. 4. Relative light intensity at various depths during 1968–69 as estimated from Secchi disc data. Data obtained with a photometer in 1964-65 included for comparison (courtesy of K. O. Emery).

1. Mean, D = 1.5 m
2. Minimum, D = 0.9 m (8/24/69)
3. Maximum, D = 2.0 m (7/2/69)

} Secchi disc

4. 6/27/64
5. 2/1/65

} photometer

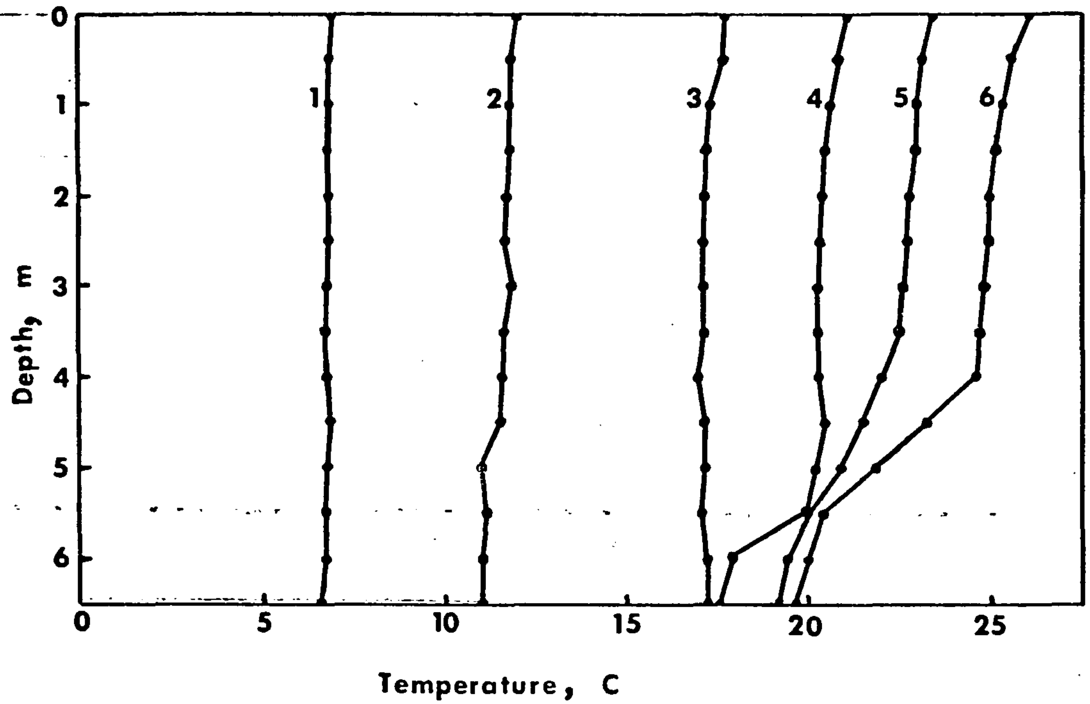


Fig. 5. Representative data showing the development of a thermal stratification during the spring and summer of 1969.

1. 3/26/69 2. 4/21/69 3. 5/21/69 4. 6/24/69 5. 7/5/69 6. 8/15/69

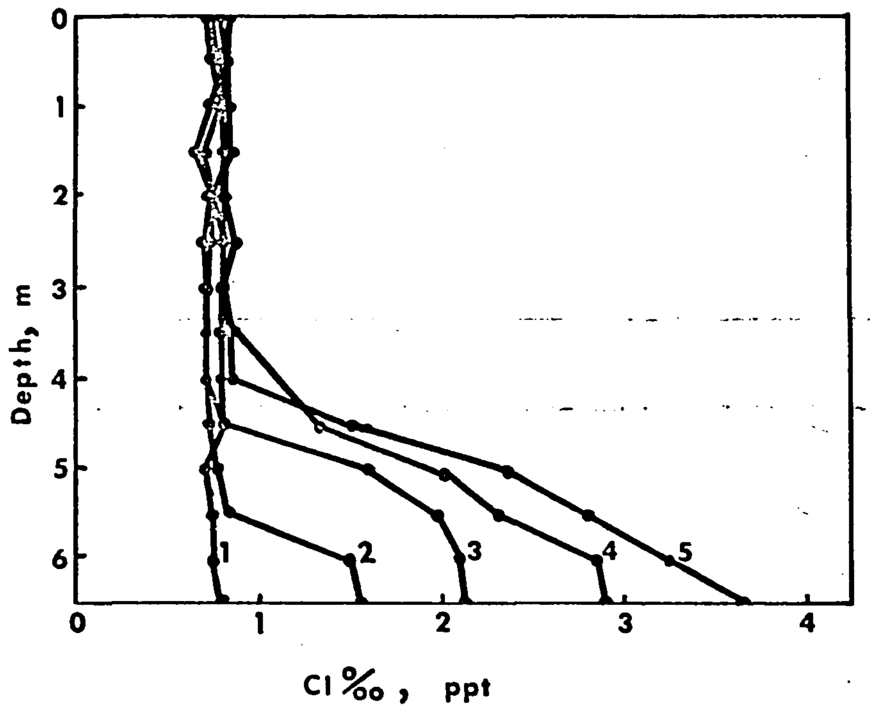


Fig. 6. Representative data showing the development of a saline stratification during the spring and summer of 1969.

1. 4/21/69 2. 6/24/69 3. 7/14/69 4. 7/27/69 5. 8/15/69

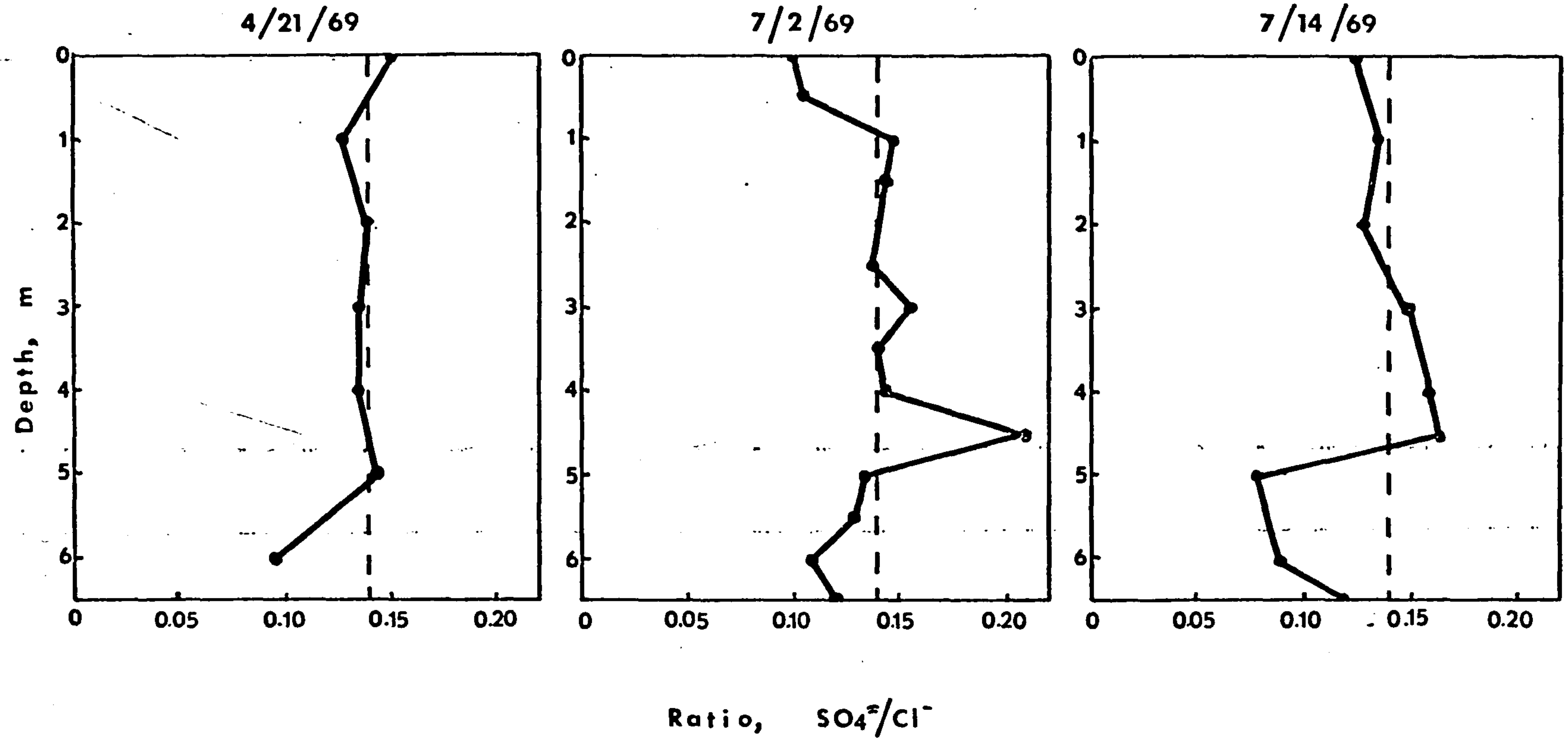


Fig. 7. $SO_4^{=}/Cl^{-}$ ratio at depths on various dates.

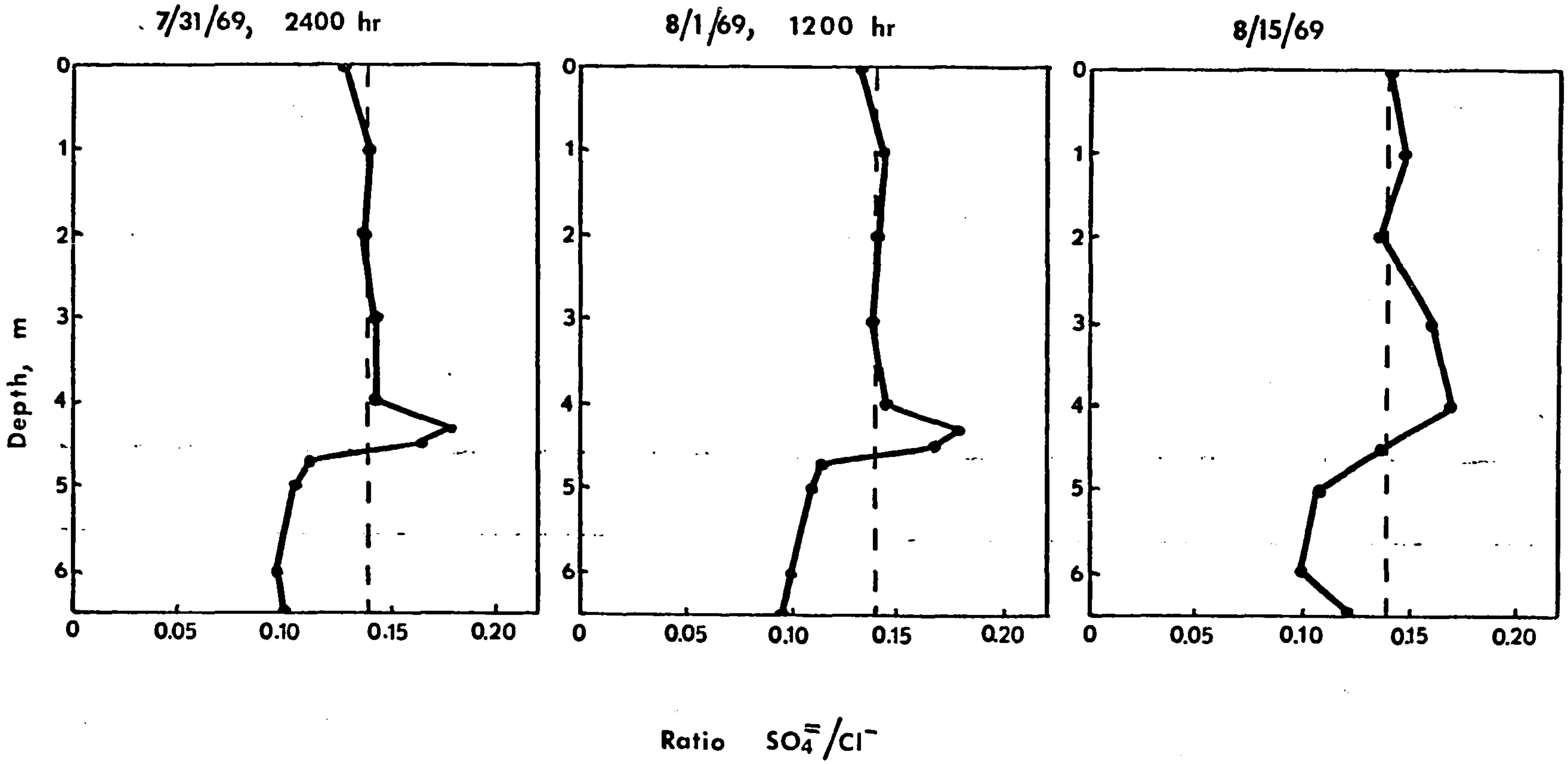


Fig. 8. $SO_4^{=}/Cl^{-}$ ratio at depths on various dates.

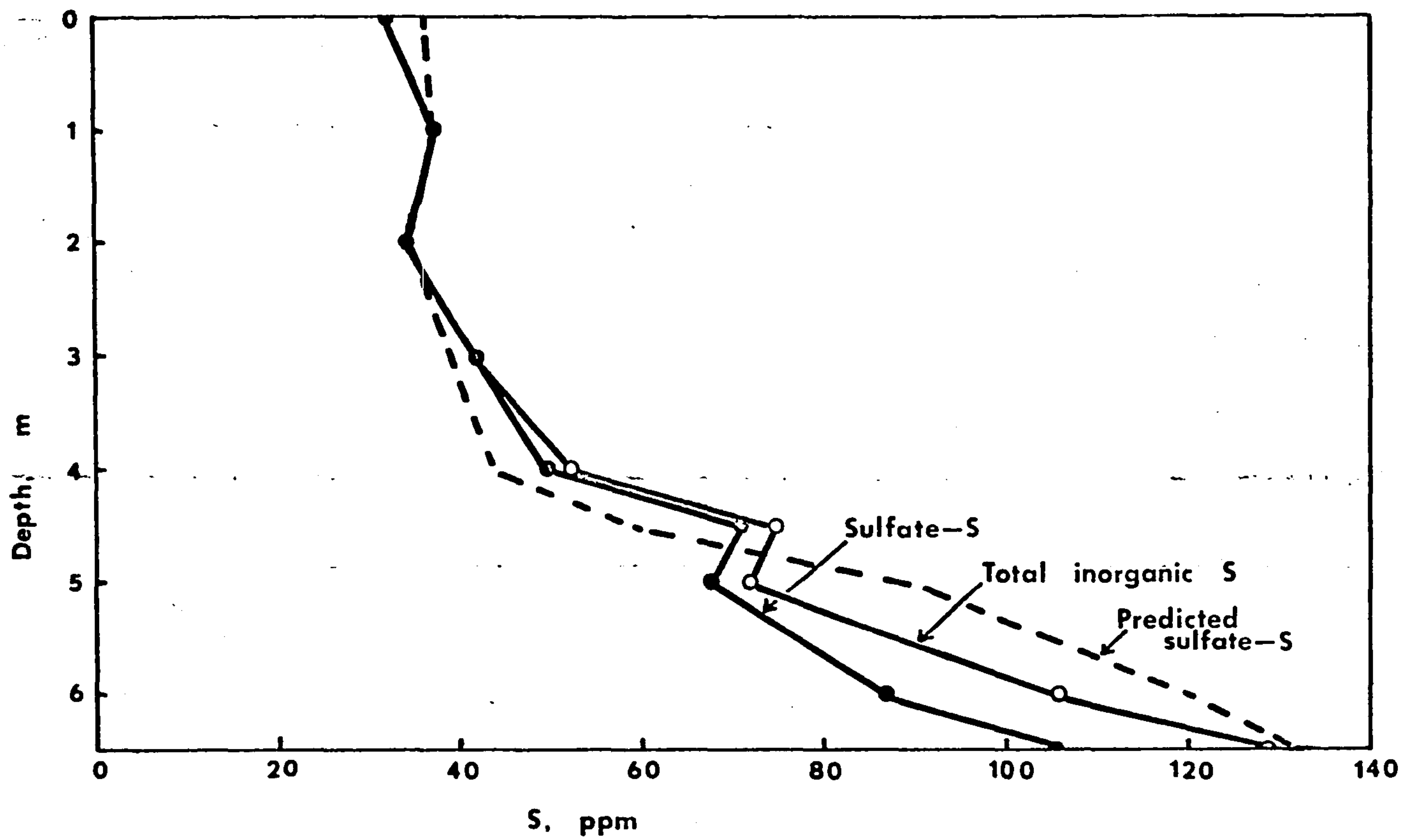


Fig. 9. Inorganic sulfur balance of water column; mean data for July and August, 1969. Sulfate − S and total inorganic S were compared with sulfate − S predicted from a seawater $SO_4^{=}$: Cl^{-} ratio of 0.1394.

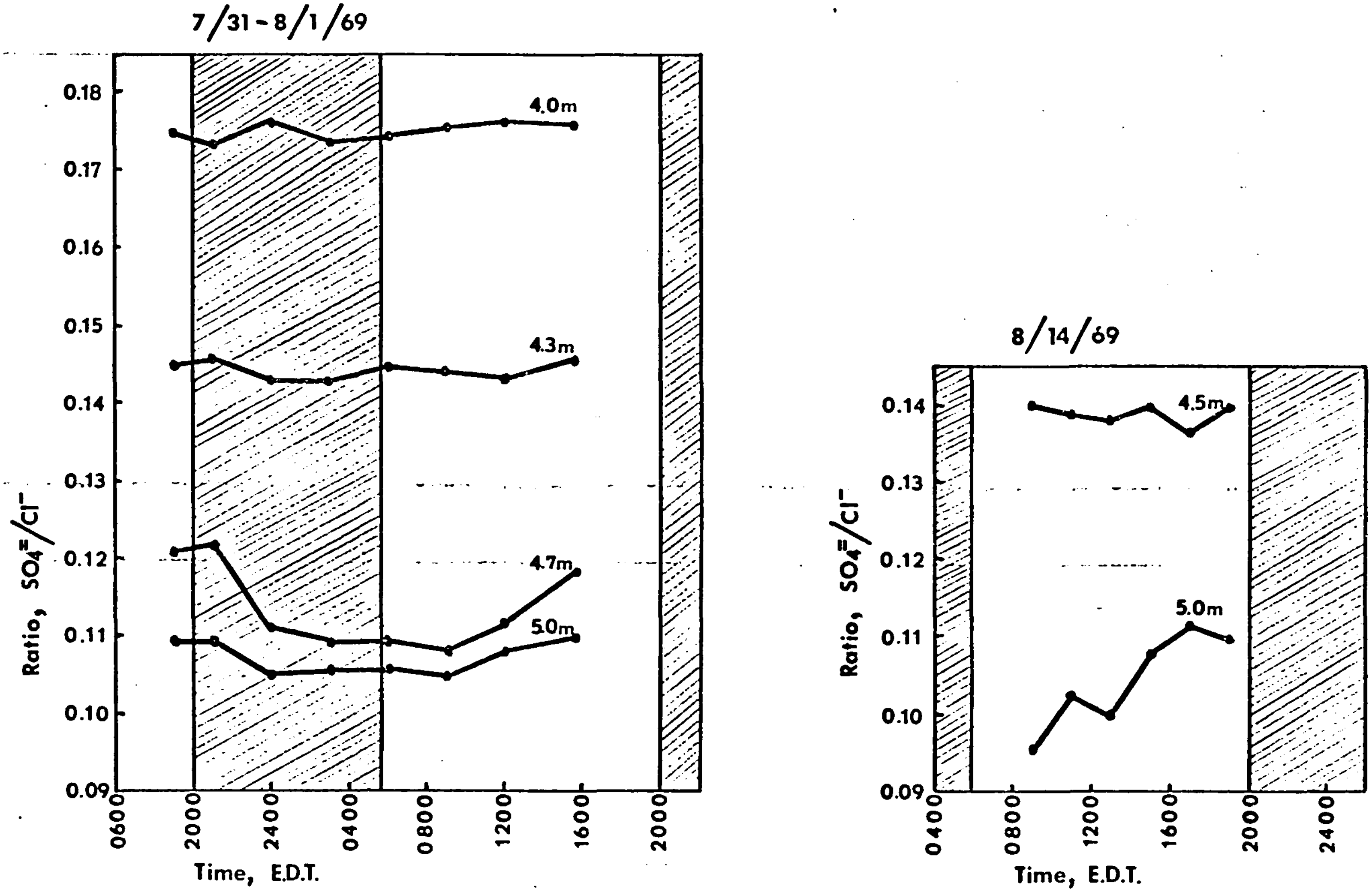
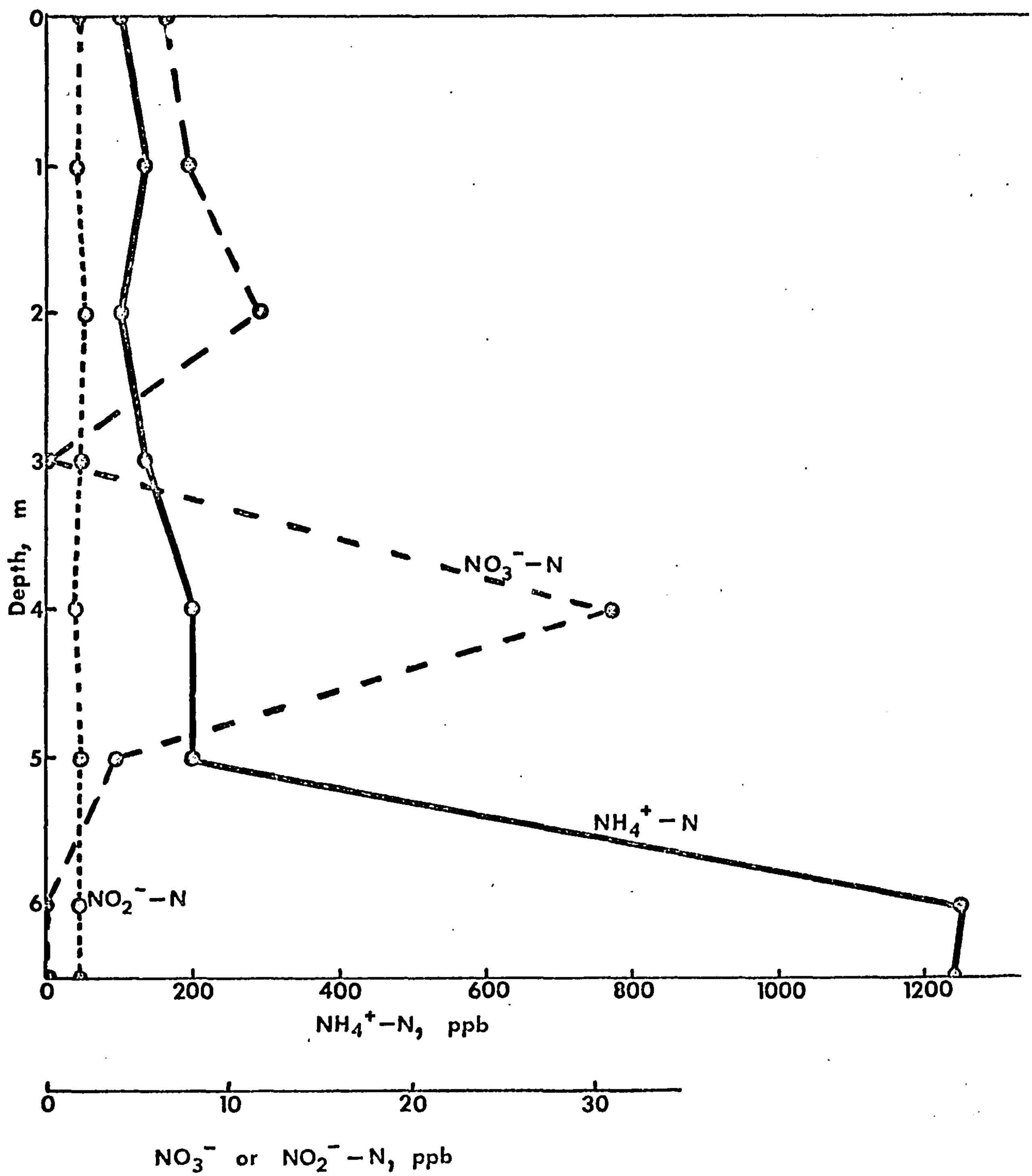


Fig. 10. Diurnal fluctuation of $SO_4^{=}/Cl^-$ ratio at various depths. Cross hatching indicates dark periods.

Fig. 11. Distribution of soluble ammonium, nitrite, and nitrate, at depths on 7/2/69.



Depth, m
0
1
2
3
4
5
6
NO3⁻ – N
NH4⁺ – N
NO2⁻ – N
0
200
400
600
800
1000
1200
NH4⁺–N, ppb
0
10
20
30
NO3⁻ or NO2⁻ – N, ppb

Fig. 12. Distribution of soluble ammonium, nitrite, and nitrate, at depths on 7/14/69.

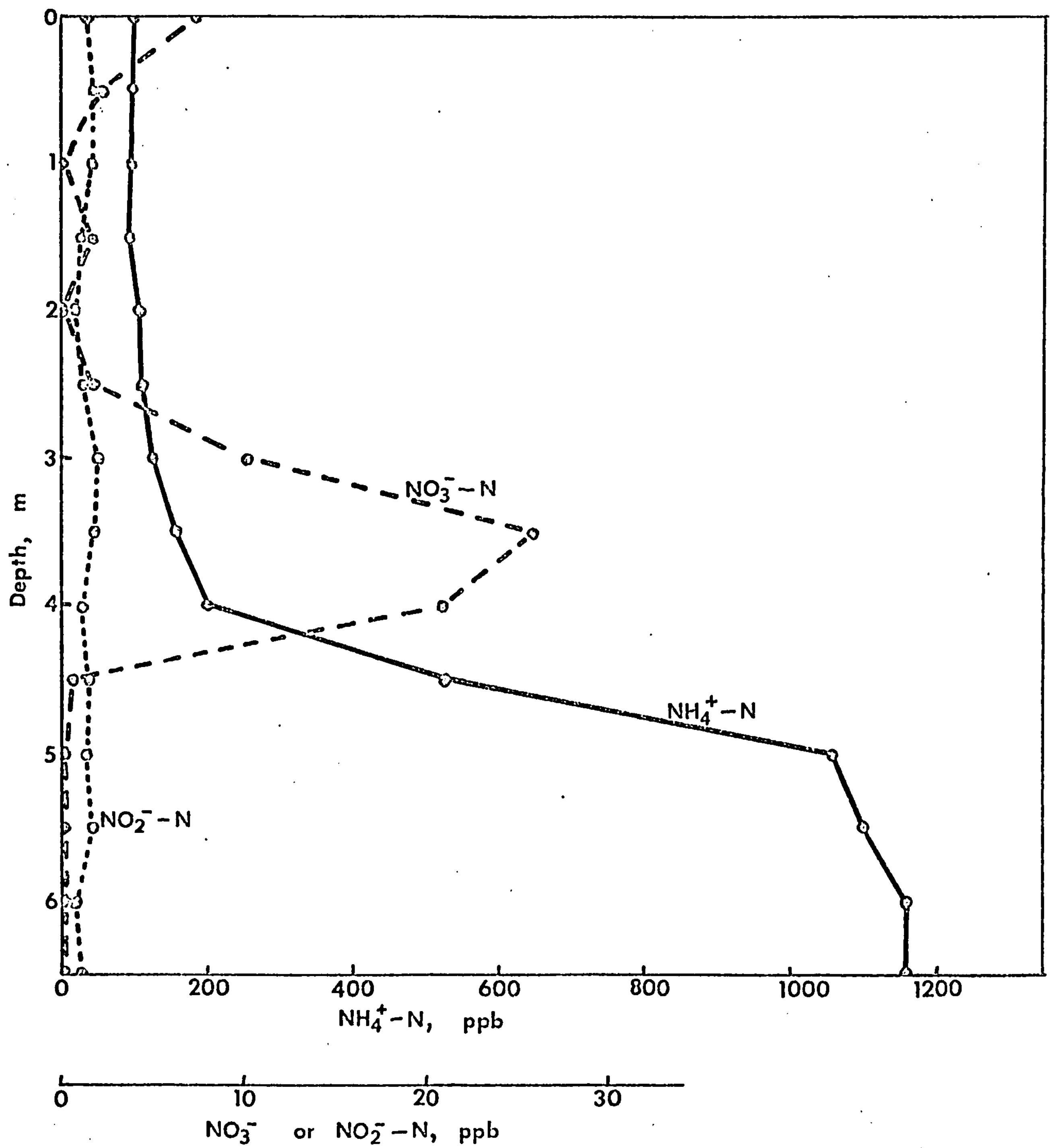
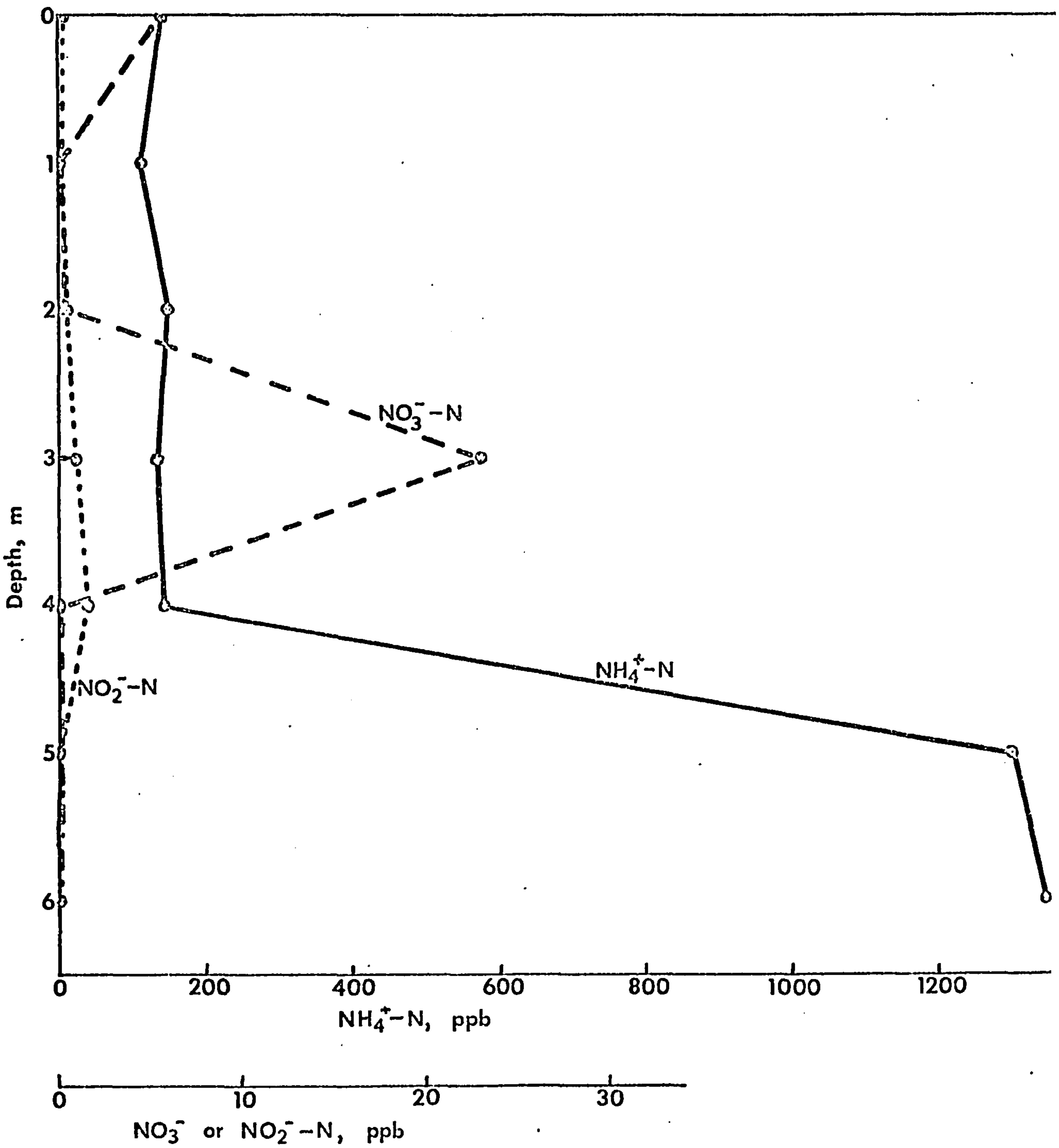

0
1
2
3
4
5
6
Depth, m
NO3⁻ – N
NH4⁺ – N
NO2⁻ – N
0
200
400
600
800
1000
1200
NH4⁺ – N, ppb
0
10
20
30
NO3⁻ or NO2⁻ – N, ppb

Fig. 13. Distribution of soluble ammonium, nitrite, and nitrate, at depths on 8/1/69.



0
1
2
3
4
5
6
Depth, m
NO$_3^-$–N
NH$_4^+$–N
NO$_2^-$–N
0
200
400
600
800
1000
1200
NH$_4^+$–N, ppb
0
10
20
30
NO$_3^-$ or NO$_2^-$–N, ppb

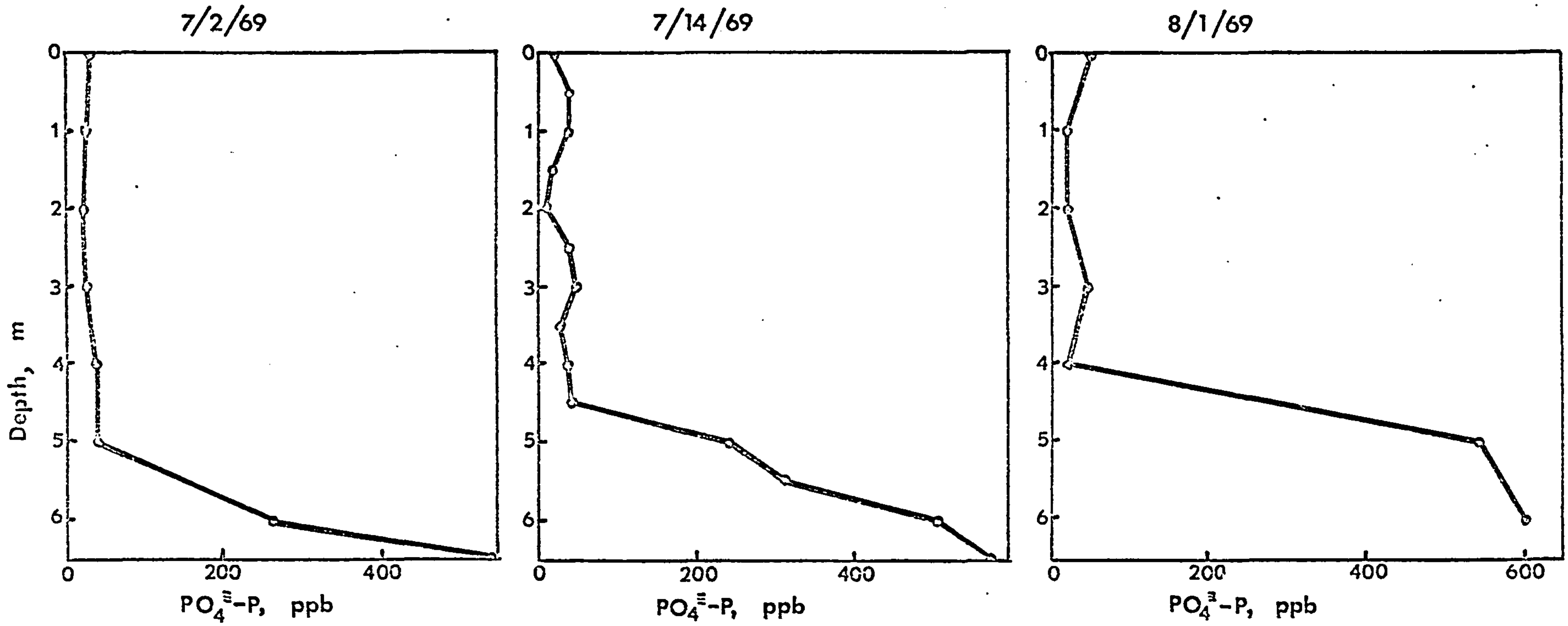


Fig. 14. Distribution of soluble reactive phosphorus at depths on 7/2/69, 7/14/69, and 8/1/69.

Fig. 15. "In vitro" and "in vivo" absorption spectra of a 12-day Chlorobium enrichment from 6.0 m, 7/15/69. "In vitro" spectrum: 10 ml culture filtered with glass fiber filter; filter extracted with 10 ml methanol; absorbance measured with 1.0 cm cuvette. "In vivo" spectrum: absorbance of original culture measured with 5.0 cm cuvette.

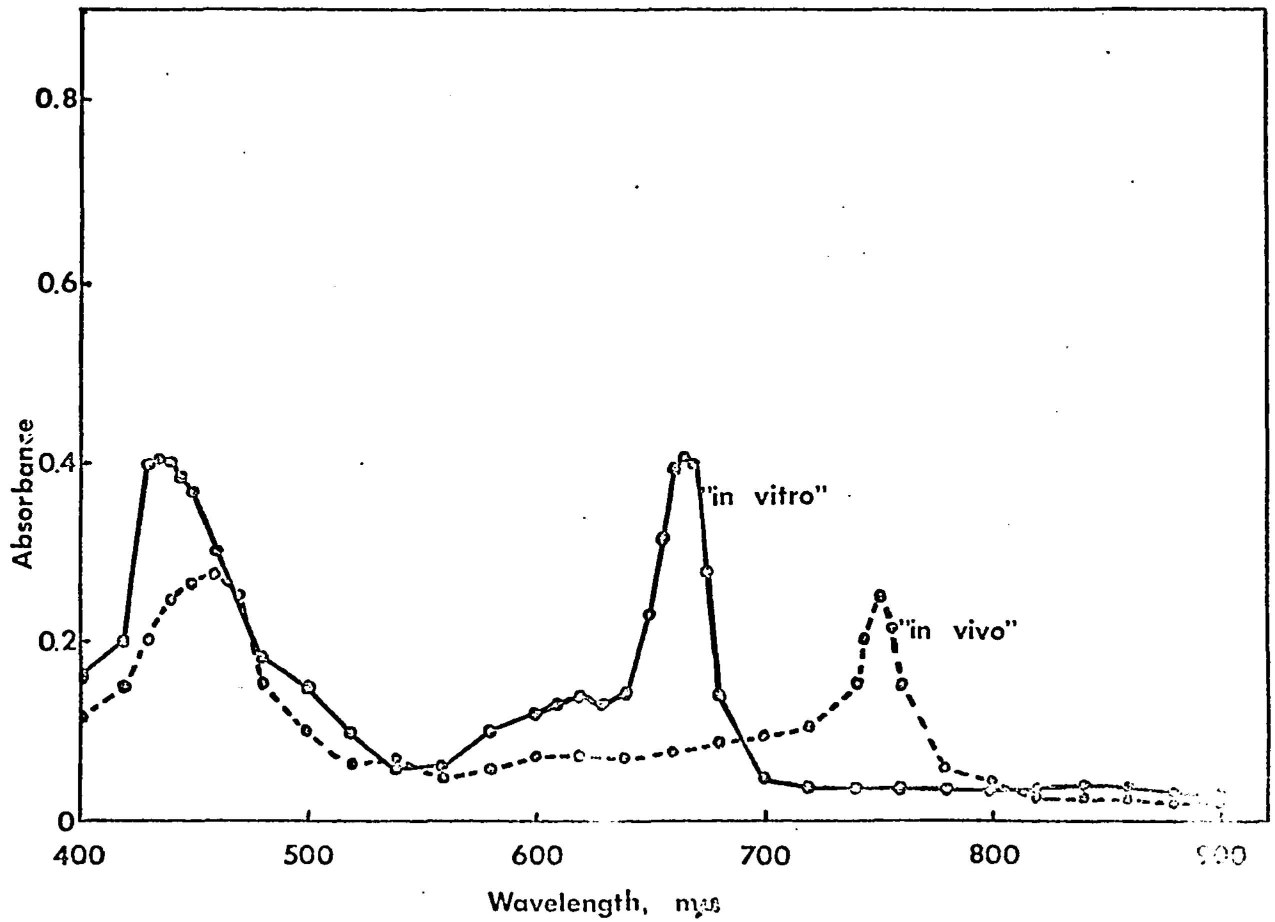

0.8
0.6
0.4
0.2
0
Absorbance
400
500
600
700
800
Wavelength, mμ
"in vitro"
"in vivo"

Fig. 16. "In vitro" and "in vivo" absorption spectra of a 12-day *Chromatium* enrichment from 5.5 m, 7/15/69. "In vitro" spectrum: 10 ml culture filtered with glass fiber filter; filter extracted with 10 ml methanol; absorbance measured with 1.0 cm cuvette. "In vivo" spectrum: absorbance of original culture measured with 5.0 cm cuvette.

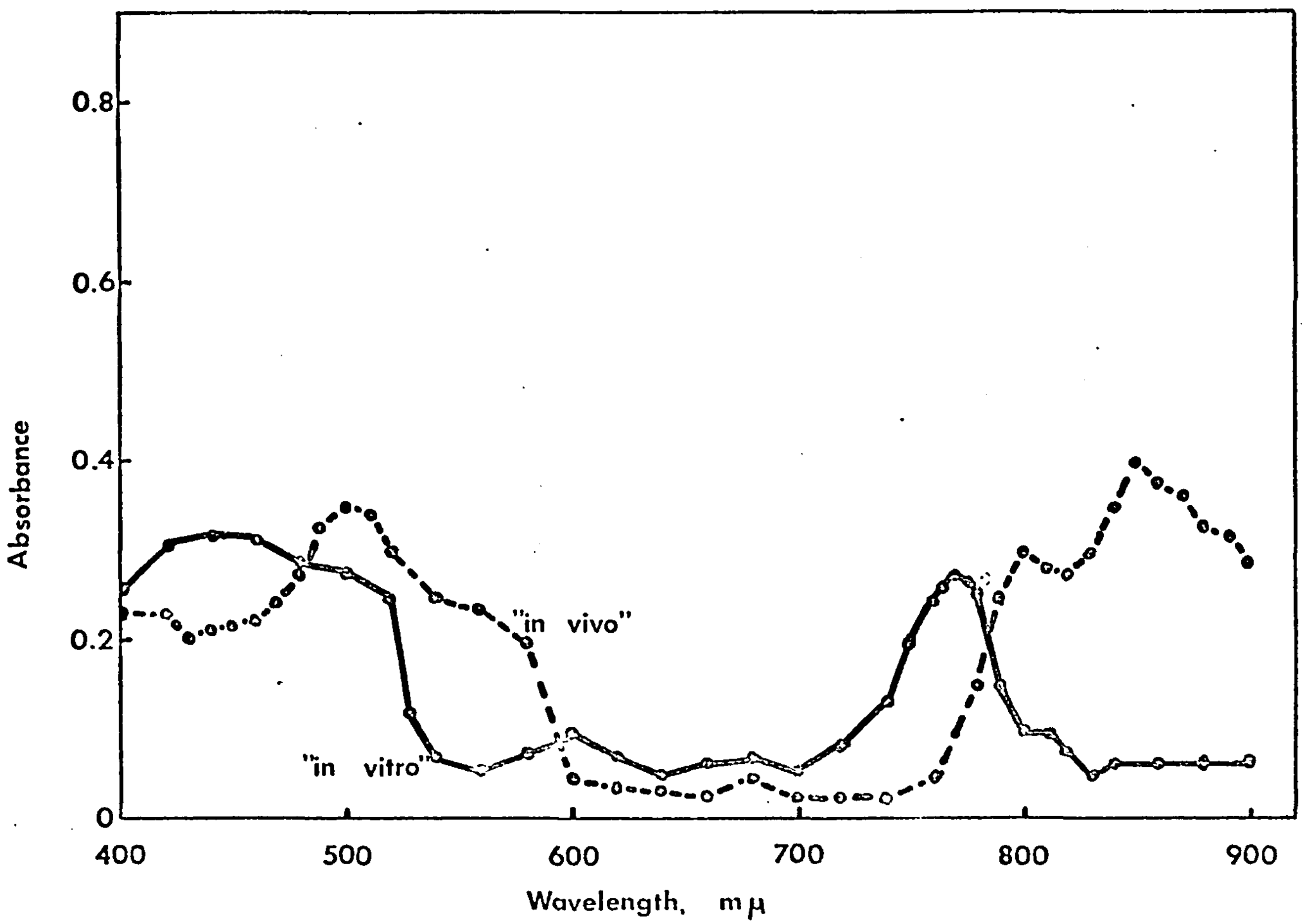

0.8
0.6
0.4
0.2
0
Absorbance
"in vivo"
"in vitro"
400
500
600
700
800
900
Wavelength, mμ

Fig. 17. "In vitro" and "in vivo" absorption spectra of water samples from 5.5 m on 8/14/69 and 8/15/69, respectively. "In vitro" spectrum: 100 ml water sample filtered with glass fiber filter; filter extracted with 10 ml methanol; absorbance measured with 1.0 cm cuvette. "In vivo" spectrum: absorbance of water sample measured with 5.0 cm cuvette.

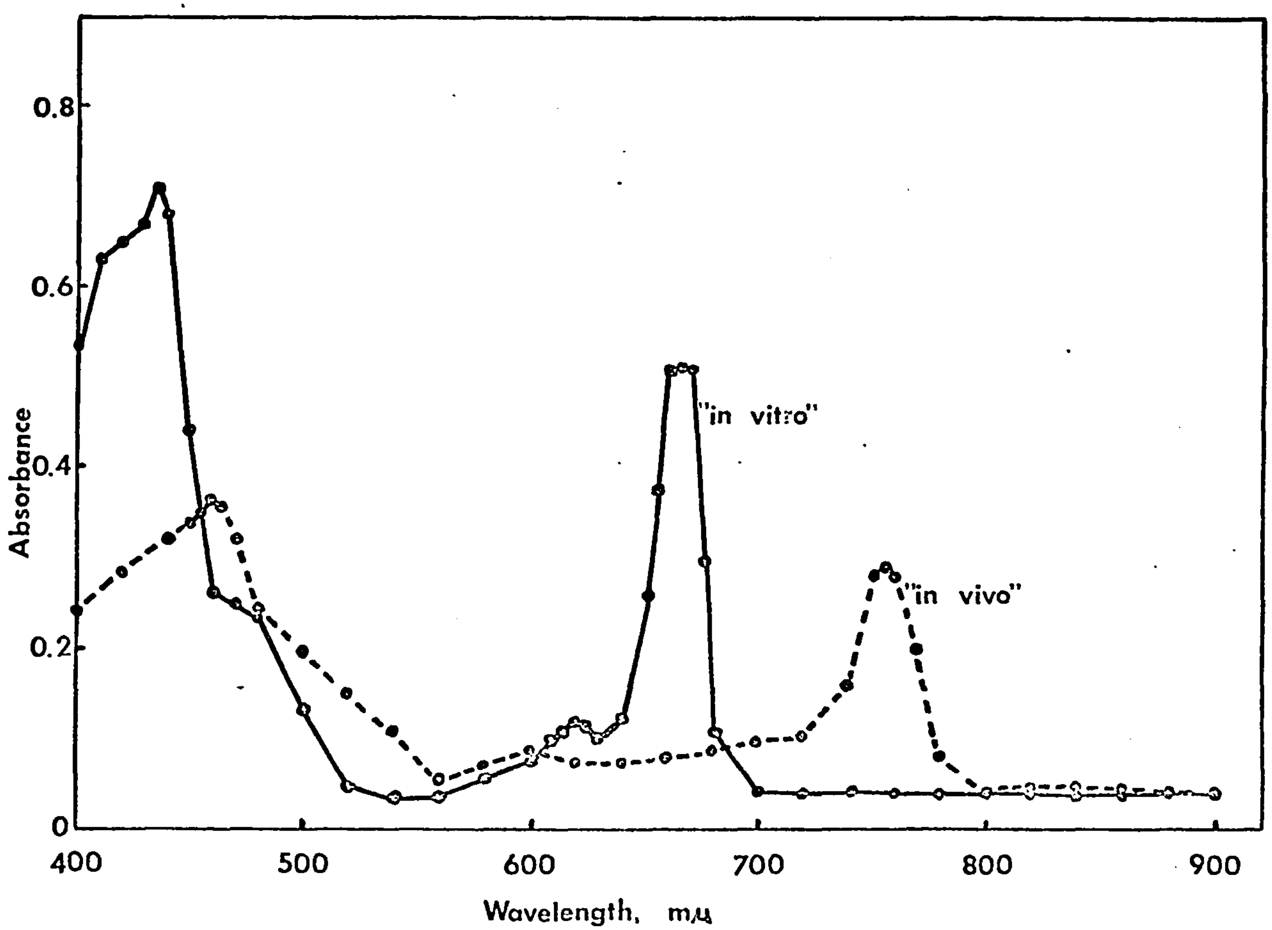

Absorbance
0.8
0.6
0.4
0.2
0
400
500
600
700
800
900
Wavelength, mμ
"in vitro"
"in vivo"

Fig. 18. Light-dark and dark productivity, measured using $NaHC^{14}O_3$, at various depths on 7/29/68, 7/19/69, 7/28/69, and 8/24/69.

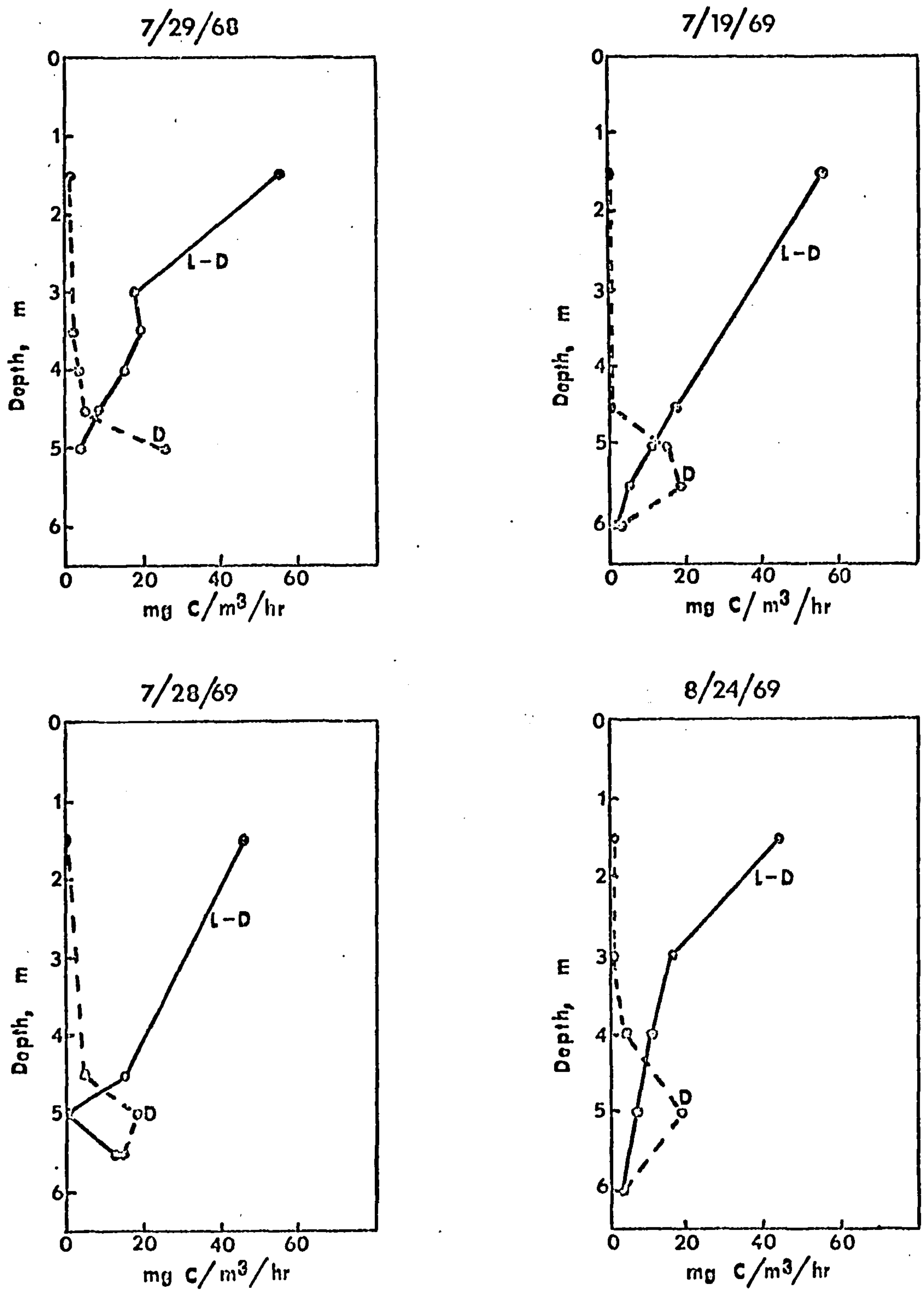

7/29/68
7/19/69
7/28/69
8/24/69
Depth, m
mg C/m³/hr
L-D
D
0
1
2
3
4
5
6
20
40
60

Fig. 19. Oxidation of S^{35}-sulfide in 24 hr in water samples collected from 1.5, 5.0, and 6.0 m on 7/5/69. Incubation in the light and dark, presence and absence of formalin.

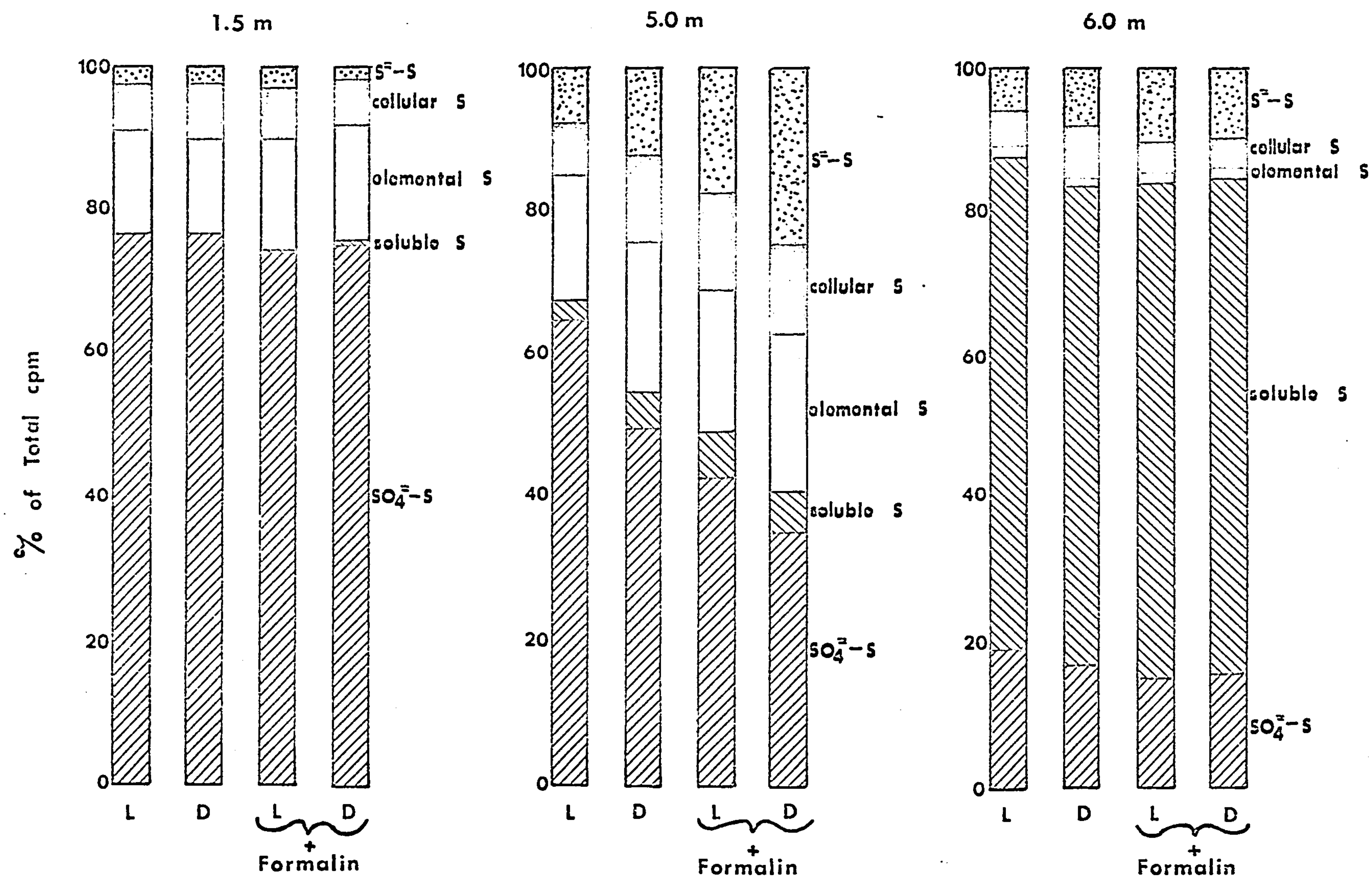
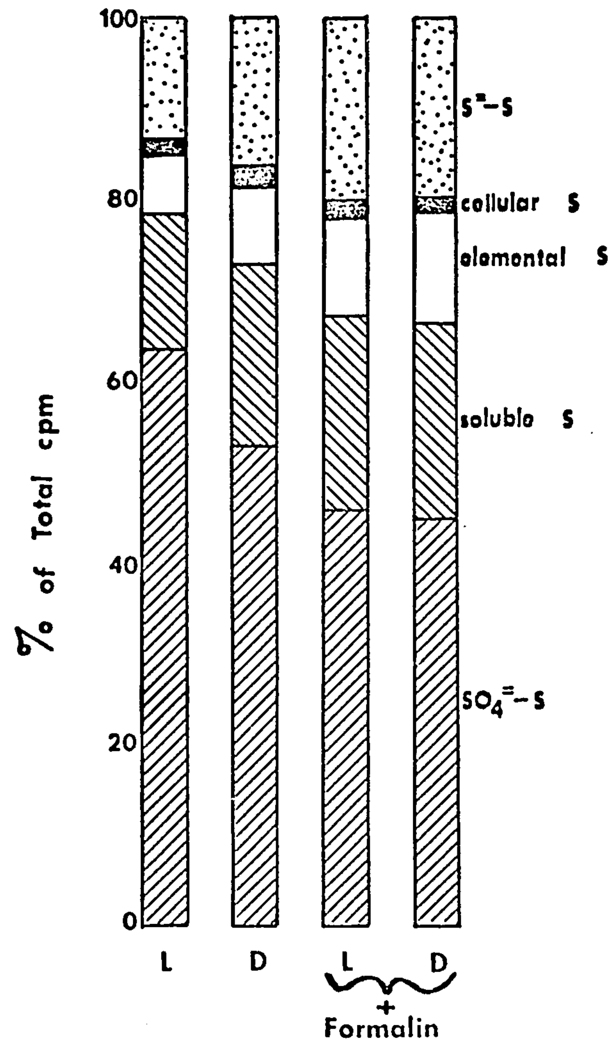

1.5 m
5.0 m
6.0 m
% of Total cpm
100
80
60
40
20
0
$S^=-S$
cellular S
elemental S
soluble S
$SO_4^=-S$
L
D
L
D
+ Formalin

Fig. 20. Oxidation of S^{35}-sulfide in 24 hr in water samples collected from 5.0 m on 8/15/69. Incubation in the light and dark, presence and absence of formalin.



100
80
60
40
20
0
% of Total cpm
$S^{=}$-S
cellular S
elemental S
soluble S
$SO_4^{=}$-S
L
D
L
D
+
Formalin

Fig. 21. Oxidation of S^{35}-sulfide with added *T. thioparus* cells in water samples collected from 6.2 m on 11/8/69. 0, 10^2, 10^4, and 10^6 cells/100 ml of a pure culture were added to samples prior to incubation in the dark at 20 C in the presence and absence of formalin.

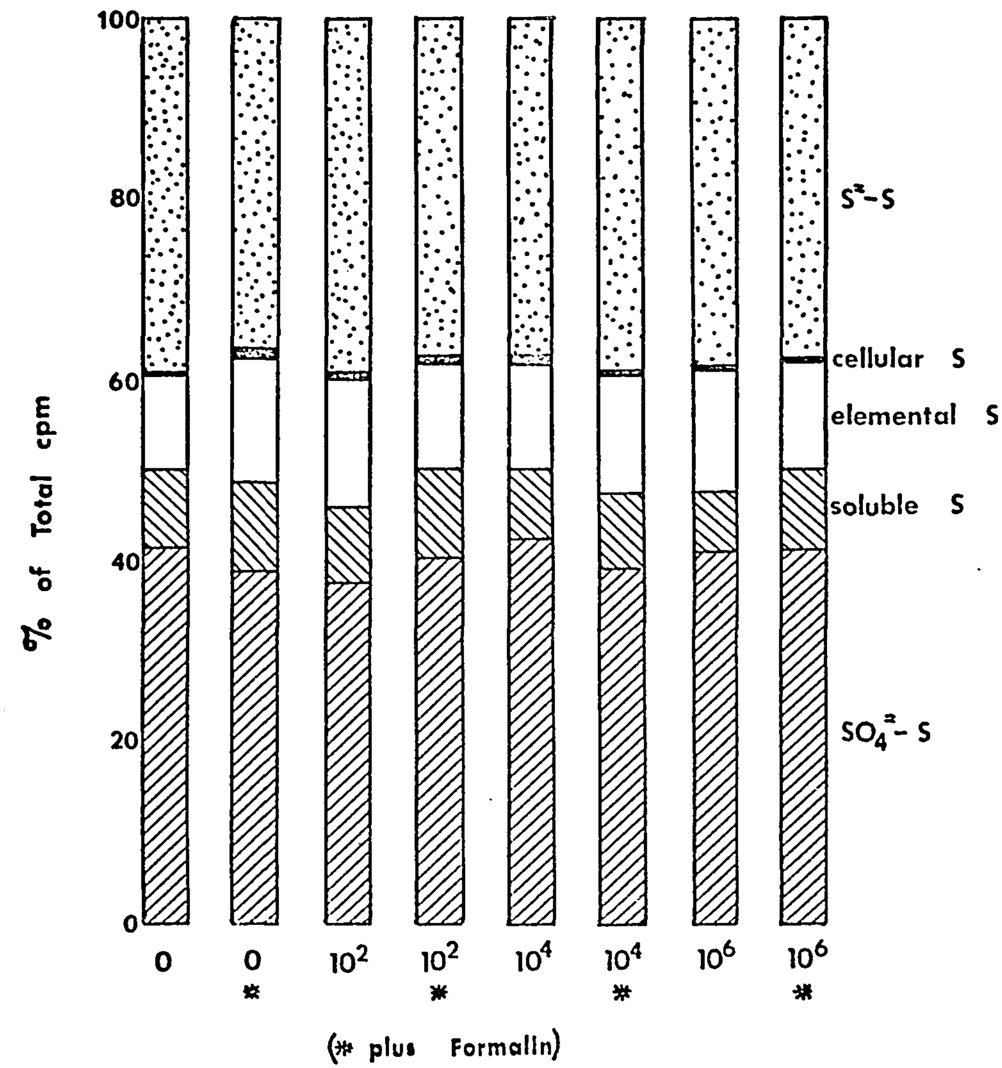
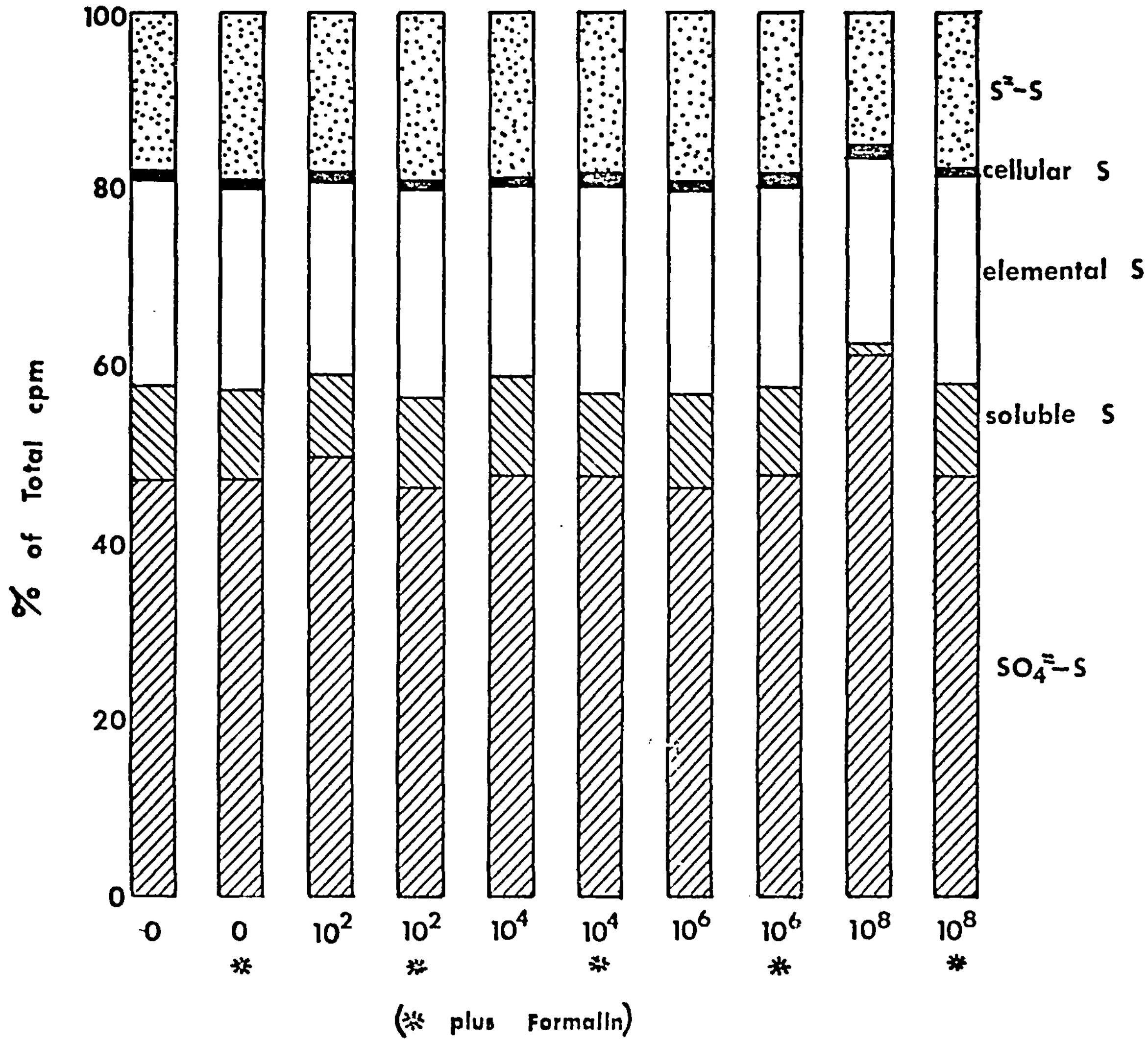

% of Total cpm
100
80
60
40
20
0
0
0 *
10^2
10^2 *
10^4
10^4 *
10^6
10^6 *
(* plus Formalin)
S=-S
cellular S
elemental S
soluble S
SO4=- S

Fig. 22. Oxidation of S^{35}-sulfide with a pure culture of T. thioparus in Basal Medium plus 1.0 ppm sulfide. 0, 10^2, 10^4, 10^6, and 10^8 cells/100 ml of a pure culture were added to sterile medium prior to incubation in the dark at 20 C in the presence and absence of formalin.



100
80
60
40
20
0
% of Total cpm
0
0 *
10²
10² *
10⁴
10⁴ *
10⁶
10⁶ *
10⁸
10⁸ *
(* plus Formalin)
S=-S
cellular S
elemental S
soluble S
SO4=-S

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